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Viruses in Water and Reclaimed Wastewater

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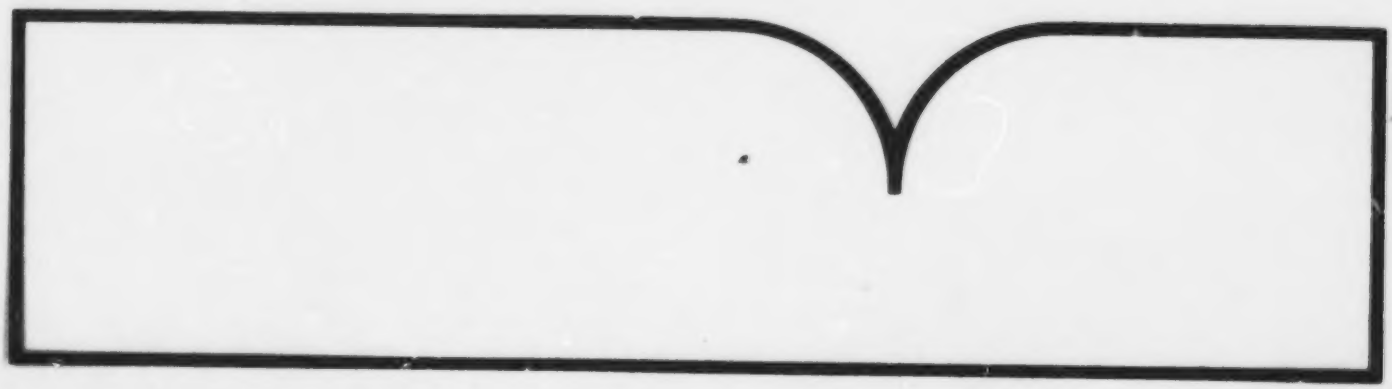

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VIRUSES IN WATER AND RECLAIMED WASTEWATER

by

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16. ABSTRACT

A study was initiated to determine the occurrence and concentration of viruses in high quality wastewater treatment plant effluents from two treatment plants in California. Disinfected secondary effluent was compared to disinfected and filtered secondary effluents. Biweekly quantitative data from the two wastewater treatment plants were obtained over a 16-17 month period. Four cell systems were used for virus assays. The BGMK, RDD and MFK cells routinely isolated 2 to 4 $\log_{10}$ plaque forming units of virus per gallon of influent. Although less sensitive, FDK cells were valuable in isolating adenoviruses. Reovirus and echovirus type 7 were the most frequently isolated viruses in this study. Viruses were isolated from the disinfected final effluent water on only two occasions. Both isolations were made from samples collected at one treatment plant. Studies were also conducted to determine the viral etiology of waterborne outbreaks of acute infectious nonbacterial gastroenteritis. During the period of this report five outbreaks of acute gastroenteritis were suspected to be of waterborne origin. Evidence of viral etiology was obtained in three of the five outbreaks. The Norwalk agent was found in stools from one outbreak. Small round viruses, 27 nm in diameter, were found by immune electron microscopy in stools from two other outbreaks. Cell cultures from chimpanzees, marmosets and monkeys were used in attempts to culture the agents producing gastroenteritis in humans. In no case was a virus isolated nor growth detected.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report provides data on the occurrence of human enteric viruses in high quality wastewater effluents that may be used to irrigate green areas where direct human contact could occur. In addition, the use of current procedures for recovering viruses from water associated with outbreaks of infectious disease was evaluated. Studies of this type provide the data base for determining the health hazard of environmental exposure to infectious agents and provide a field evaluation of the methodology developed for their detection.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

A study was initiated to determine the occurrence and concentration of viruses in high quality wastewater treatment plant effluents from two treatment plants in northern California. Disinfected secondary effluent from the San Pablo treatment plant was compared to disinfected and filtered secondary effluents from the Livermore treatment plant. Biweekly quantitative data from the two wastewater treatment plants were obtained over a 16-17 month period. The identity of the viral isolates and the sensitivity of four different cell lines in permitting the growth and isolation of the various virus types were also determined. Approximately the same number of viruses per gallon were isolated from the influents obtained from the Livermore and San Pablo treatment plants. Viruses were isolated from the disinfected final effluent water on only two occasions. Both isolations were made from samples collected at the San Pablo treatment plant.

The four cell systems used for virus assays were Buffalo green monkey kidney (BGMK), human rhabdomyosarcoma (RD), monkey fetal kidney (MFK) and human fetal diploid kidney (HFDK) cells. The BGMK, RD and MFK cells routinely isolated 2 to 4 $\log_{10}$ plaque forming units of virus per gallon of influent. Although less sensitive, the human fetal diploid kidney cells were valuable in isolating adenoviruses. The viruses recovered in this study were echovirus, poliovirus, coxsackievirus group A, coxsackievirus group B, reovirus and adenovirus. Reovirus and echovirus type 7 were the most frequently isolated viruses in this study.

Studies were also conducted to determine the viral etiology of waterborne outbreaks of acute infectious nonbacterial gastroenteritis. During the period of this report five outbreaks of acute gastroenteritis were suspected to be of waterborne origin. A field investigation group consisting of sanitary engineers, virologists, microbiologists and medical epidemiologists was established to correlate information on the viral content of suspected water, the effectiveness of water treatment processes, epidemiologic data and relevant data pertaining to any suspected waterborne outbreak of disease. Evidence of viral etiology was obtained in three of the five outbreaks. The Norwalk agent was found in stools from one outbreak. Small round viruses, 27 nm in diameter, were found by immune electron microscopy in stools from two other outbreaks.

Cell cultures from chimpanzees, marmosets and monkeys were used in attempts to culture the agents producing gastroenteritis in humans. Such techniques as centrifugation of inoculum onto cells and treatment of cells with dilute solutions of trypsin were used separately and in combination. Evidence of growth was sought by immunofluorescence staining of inoculated cultures with convalescent-phase human serum from individuals with

gastroenteritis who were found by immune electron microscopy to shed 27 nm virus. In no case was a virus isolated nor growth detected by these techniques.

This report was submitted in fulfillment of Grant Number R806563010 by the Health Effects Research Laboratory under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period April 23, 1979, to April 22, 1981, and work was completed as of April 30, 1981.

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We would like to thank the West Contra Costa Sanitary District and the City of Livermore for allowing us to use their facilities during the course of the study. We greatly appreciate the cooperation and assistance we received from William Kennedy, superintendent, and his staff at the West Contra Costa Sanitary District's Water Pollution Control Plant and John Deponte, supervisor, William Loftin, former superintendent, and William Adams, superintendent, and their staff at the City of Livermore's Water Pollution Control Plant.

INTRODUCTION

In recent years there has been increasing emphasis in the U.S. on conserving and reusing water to the greatest extent possible. The reuse of wastewater effluents has become an integral part of the overall management of water resources. In addition, there has been strong support at the federal level to use the concepts of land treatment and disposal as viable alternatives to ocean and stream discharge of wastewater effluents. There are no Federal regulations defining treatment requirements and water quality criteria for wastewater reuse. Although several states have established reuse regulations, they differ significantly from state to state.

California has a long history of wastewater reuse and has had, with periodic updating, reclamation criteria since 1918. California also has more variety of wastewater uses than probably any other state. Historically most reuse activities have involved agricultural irrigation of non-food crops where the health risks are low. In the last few years, however, reuse has expanded to the point where effluents are being used to irrigate golf courses and other broad landscape areas, for food crop irrigation, and to develop recreational impoundments for boating, fishing, and swimming. In addition, wastewater effluent has been proposed as a secondary water source for the irrigation of urban parks, playgrounds, schoolyards, and around residential homes.

With the expansion in wastewater reuse will come an increase in public exposure and risk to infectious agents, particularly viral pathogens. The purpose of this aspect of the study was to develop sufficient information on viruses in reclaimed wastewaters in order to assess the public health risks posed by the presence of such viruses in the reused wastewater.

OBJECTIVES

Objective No. 1, was to determine the occurrence and concentration of viruses in high quality wastewater treatment plant effluents. As stated in the original proposal, the increased emphasis placed on the reuse of wastewater effluents for purposes of irrigation and recreation presents varying degrees of health risks which are commensurate with varying levels of wastewater treatment, water quality, and public exposure. The concern from a health standpoint where public contact with wastewater effluents occur is the spread of infectious diseases. Most bacterial pathogens can be controlled with adequate disinfection practices; however, very little is known whether such practices can adequately control viral pathogens.

Costly advanced waste treatment processes have been required where virus control was necessary, but with increasing emphasis on land treatment and

disposal practices, less costly treatment approaches are being proposed. Although existing California wastewater reuse criteria are among the most stringent in the United States, there is little information on levels of viruses in effluents which meet these criteria. One of the main objectives of this study was to obtain quantitative data on virus concentrations in high quality effluents such as well disinfected secondary effluents, as compared with disinfected, filtered secondary effluents.

Objective No. 2. was the detection and identification of viruses in waters which are suspected sources of infection in waterborne disease outbreaks. A field investigation group consisting of sanitary engineers, virologists, microbiologists and medical epidemiologists was established to correlate information on the viral content of suspected water, the effectiveness of water treatment processes, epidemiologic data, and other relevant data pertaining to any suspect waterborne outbreak of diseases. Virus concentrating equipment and personnel for sampling large volumes of suspected water immediately after an outbreak were to be provided. The assay of the samples in the laboratory for the presence of specific causative viruses was then to be carried out.

Objective No. 3. was to develop and evaluate cell culture technics for isolation of viral gastroenteritis agents from cases and outbreaks of suspected viral diarrhea. In certain instances attempts were made to isolate viral gastroenteritis agents from fecal specimens from cases and outbreaks of suspected viral diarrhea. Although less emphasis was placed on this objective, different methods of treatment were used to enhance cell susceptibility for growth of viral agents.

CONCLUSIONS & RECOMMENDATIONS

Viruses were detected in treated wastewater effluents only twice during the course of the study. Possibly the organic load of the effluent water competes with viruses for adsorption sites on the pleated membrane filters when utilizing this technique of virus concentration.

Different cell systems are sensitive to different viruses as was shown in the study and any type of proposed regulations concerning viruses in water will have to be qualified as to the kind of viruses detected and allowed.

In three of five outbreaks of waterborne gastroenteritis investigated and studied, a parvovirus-like particle was implicated as the causative agent.

INVESTIGATIVE APPROACHES

WASTEWATER TREATMENT PLANT STUDY

Permission was obtained from the city of Livermore, California and the city of San Pablo, California to allow sampling at their wastewater treatment plants to assay for viruses. The plant at San Pablo produces a well disinfected secondary effluent, while the plant at Livermore produces a disinfected, filtered secondary effluent. Both plants neutralized the chlorine used for disinfection before releasing the final effluent. Schematic flow diagrams of the two plants are shown in Fig. 1 and Fig. 2. A schedule of sampling was set up such that the treatment plant at San Pablo and the Livermore plant were sampled every other week; thus a weekly concentration and assay procedure was carried out.

Virus Concentration

The equipment, instruments and personnel needed for the concentration procedures were transported to the collection site in a van. The concentration procedure consisted of pumping the sample water (usually 100 gal.) through an orlon pre-filter or a sand pre-filter and then to a proportional pump which added $AlCl_3$ to 0.0005 M and adjusted the pH to approximately 3.5. The sample was then pumped through 0.45µm "Filterite" filters at a rate of approximately 2 gal/min. A 1-4 liter sample of plant influent was also collected (usually as a 24 hr composite sample) on the day of concentration sampling. The collected samples were transported back to the laboratory on wet ice.

Laboratory Procedures

In the laboratory the "Filterite" filters were eluted with one liter of 0.05 M glycine buffer, pH 9.0, containing 0.1% beef extract. The pre-filters were eluted with one liter of 1% beef extract and the composite sample adjusted to 1% beef extract.

The eluate from the Filterite was adjusted to a pH of approximately 7.0 and further concentrated to approximately 30 ml using a Millipore "Pellicon" membrane concentrator. The eluates from the pre-filter and the composite sample were adjusted to a pH of 3.5 and allowed to floc. The floc was recovered by centrifugation and dissolved in 0.15 M Na_2HPO_4; the resulting volume was approximately 30 ml.

The samples were inoculated onto human fetal diploid kidney cells (HFDK) grown in culture tubes (5 tubes per dilution, 3 dilutions) following the MPN

procedure of Chang et. al.* The samples were also inoculated for plaque formation onto RD cells, buffalo green monkey kidney cells (BGMK), and monkey fetal kidney cells (MFK) grown in Petri plates (5 plates per dilution, 3 dilutions). The inoculum consisted of a 0.5 ml volume of the virus concentrate per culture dish to tube. Thus, approximately one half of each virus concentrate sample was inoculated onto the cell culture systems. The cultures were incubated at 36°C for from 10 to 14 days after inoculation. The plates were scored as in the MPN determination (for example, if 3 of the 5 plates each had one or more plaques, the plates were scored as 3 of 5 infected; if one plate of the five had one or more plaques with the remainder showing no plaques, the plates were scored as 1 of 5 infected, etc.) and the plaques were also counted for a total virus count. Thus an MPN determination and a total plaque count can be carried out on the same cultures. Plaques were picked and passed for confirmation, and certain of the isolates were identified serologically, using the LBM serum pools or serum pools available in our laboratories.

INVESTIGATION OF SUSPECTED WATERBORNE VIRUS DISEASE OUTBREAKS

The procedure followed in investigating gastroenteritis outbreaks was for county health officials to notify the Infectious Disease Section that such an outbreak was occurring and the Infectious Disease Section in turn provided epidemiologic assistance in carrying out the field investigation. When waterborne disease is suspected, the Department's Sanitary Engineering staff is also immediately apprised and the investigation conducted jointly by the California Department of Health Services, Sanitary Engineering Section, and the medical epidemiology staff of the Infectious Disease Section.

During suspected waterborne virus disease outbreaks our laboratory would provide for the immediate use of large volume virus concentrators to sample the suspect waters and the assay of the concentrated samples in the laboratory for the presence of specific causative viruses utilizing the same procedures as are used for the concentrated wastewater samples. At the same time fecal specimens and acute and convalescent phase serum samples were to be obtained from patients who had become infected during the outbreak. The fecal specimens would be utilized in enteric virus isolation procedures while the serum samples would be utilized in immune electron microscopic procedures if warranted.

* Chang, S. L., G. Berg, K. A. Busch, R. E. Stevenson, N. A. Clarke, and P. W. Kabler. Application of the "Most Probable Number" Method for Estimating Concentrations of Animal Viruses by the Tissue Culture Techniques. Virology, 6: 27-42, 1958.

RESULTS

WASTEWATER TREATMENT PLANT STUDY

Table 1 shows the water quality data of the effluent water for the two treatment plants during the extent of the study (a total of 63 concentration and assay procedures, 33 at San Pablo and 30 at Livermore). These data were made available to us by personnel at the plants with the exception of the coliform determinations which were assayed by our Sanitation and Radiation Laboratory. During 1980 (run no. 38) assays for fecal streptococcus were instituted on the effluent water.

Figures 3 through 6 are chronological bar charts of the variation of natural viruses in the influent of the San Pablo treatment plant as assayed on the four different cell systems (BGMK, RD, MFK, and HFDK). Depending on the cell system used, in general a late summer-early fall increase in the number of viruses isolated is noted the first year, although this was not evident during the second year of the investigation. The BGM, RD, and MFK cell systems routinely picked up from 2 to 4 Log_{10} plaque forming units of virus per gallon of influent. The HFDK cell system was less sensitive, showing usually 2 Log_{10} of virus by the MPN technique. The HFDK cell system is valuable, however, in isolating some types of viruses that grow only in that cell system (i.e. adenoviruses) of the four used.

Figures 7 through 10 are chronological bar charts of the variation of natural viruses in the influent of the Livermore treatment plant as assayed on the four different cell systems. Approximately the same number of viruses per gallon was isolated from the Livermore plant as from the plant at San Pablo. A late summer-early fall peak of virus activity was evident only in the case of the HFDK cell system. The other three cell systems showed no consistent pattern.

Figures 11 through 14 are chronological bar charts of the number of viruses isolated at the San Pablo treatment plant per gallon of treated effluent water before disinfection as assayed on the four different cell systems. Figures 15 through 18 are similar for the Livermore treatment plant. Although differences in number of viruses isolated are apparent between different cell systems used for assay, there is actually very little difference shown in the total number of viruses isolated from the pre-chlorinated effluents at the two plants.

Viruses in Final Effluents

On only two occasions (run no. 7 (79-13) and run no. 14 (79-20)) were viruses isolated from the disinfected final effluent water. Both isolations

were made from samples collected at the San Pablo treatment plant, the first in August 1979, when 0.099 MPN (1/5 undilute infected, 0/5 1:4 dilution infected, 0/5 1:16 dilution infected, Filterite filter) per gallon was isolated in the BGMK cell system and was subsequently identified as reovirus type 2. The second instance was in October 1979, when a total of 0.192 MPN per gallon was isolated in the HFDK cell system (0/5 undilute infected, 1/5 1:4 dilution infected, 0/5 1:16 dilution infected, Prefilter; 1/5 undilute infected, 0/5 1:4 dilution infected, 0/5 1:16 dilution infected, Filterite filter). These viruses were identified as echovirus type 7.

Virus Identification

Human enteric viruses (ECHO, polio, coxsackie A, oxsackie B, and reoviruses) in addition to adenoviruses were the only viruses recovered in this study. All viruses in the pre-disinfected final effluent from the two plants were identified, but because of the number of viruses isolated from the plant influent water, only representative isolates were identified. The isolates which were identified, however, probably reflected rather closely the enteric virus activity which was occurring in the general population. Table 2 shows the enteroviruses (ECHO, polio, and coxsackie B virus) isolates at the two plants ranked according to the frequency of isolation. Echoviruses were the predominant subgroup of enteroviruses isolated with echovirus type 7 being the most frequently isolated enterovirus at both the Livermore and San Pablo treatment plants.

Reovirus was the most frequently isolated virus group during the course of the study. Isolates were passed in cell culture and were identified as reoviruses by immunofluorescence. Reoviruses were isolated in 42 of the 63 different runs (67%). A total of 534 different reovirus isolates were obtained and identified during the study period. Randomly selected isolates were typed throughout the study period and of 71 different isolates, 66 were reovirus type 2, 4 were reovirus type 3 and 1 isolate was reovirus type 1.

Table 3 shows the number of coxsackie A virus types isolated in the four different cell culture systems used. A total of 146 coxsackie A viruses were isolated during the study consisting of 15 different types. Twelve different types were isolated in the RD cell system, 7 in the MFK, 6 in the HFDK, and 2 in the BGMK system. Five types of coxsackie A virus were isolated only in the RD cell system, 1 only in the MFK cells, 2 only in the HFDK cells while no coxsackie A viruses were isolated only in the BGMK cells. The RD cell culture system was the most sensitive to these viruses in that over 80% of the isolates were obtained in this cell system.

Adenoviruses were also isolated at the two treatment plants. Figures 19 and 20 are bar charts of the numbers and types of adenoviruses isolated during the study. Isolates were identified as adenovirus by immunofluorescence and were then typed by neutralization techniques using immune sera available in our laboratories. In several instances serologically intermediate strains were isolated (4/16, 11/21, and 15/29). These were not confined to any one plant but were isolated from both San Pablo and Livermore.

SUSPECTED WATERBORNE VIRAL DISEASE OUTBREAKS

HUMBOLDT COUNTY

In September of the first year of the study a possible waterborne outbreak of acute gastroenteritis occurred in the city of Arcata, Humboldt County, California. Thirty members of a little league football team, while at a practice session, drank water from spray irrigators being used to water portions of the football field. The water was being pumped from a small creek flowing near the football field. Symptoms of acute gastroenteritis (vomiting, diarrhea) occurred in all 30 of those drinking the water, while 13 individuals who did not drink the water remained symptom-free. Stool samples and acute and convalescent serum samples from several individuals were obtained and submitted to our diagnostic laboratory for study. Some time after the outbreak, a sample of one gallon of the creek water was submitted to the water virology unit for assay. After organic flocculation to concentrate any viruses present, the resulting concentrate was inoculated onto RD and BGMK cells in an MPN type assay. No viruses were isolated in the RD cell cultures, but in the BFMK cells approximately [illegible] viruses per gallon were obtained. Additional cultures of RD and BGMK cells were inoculated with aliquots of the concentrates from the water by high speed centrifugation. The RD cell system detected a total of approximately 19 viruses per gallon of water, while the BGMK cell system detected a total of >1,566 viruses per gallon, again showing the different sensitivity of the two cell systems to the different viruses present. Representative samples of the isolates which showed reovirus CPE were identified as reoviruses by IFA and some were typed, all proving to be reovirus type 2. The isolated viruses which showed enterovirus CPE were subsequently typed as echovirus types 11, 13, and 25.

The stool and serum samples submitted to the laboratory were examined by Dr. Lyndon Oshiro by immune electron microscopy (IEM). Two of six stool samples submitted showed a parvovirus-like particle to be present, while 11 of 16 convalescent serum samples reacted with the particles. None of the acute serum samples reacted with the parvovirus-like particles to any extent and it is assumed that the particles present were the cause of the gastroenteritis.

The creek from which the irrigation water was obtained was subsequently shown to be contaminated by a leaking sewer line.

SODA SPRINGS

In January 1980 a possible waterborne outbreak of acute gastroenteritis occurred in the Serene Lakes district, which is a small community adjacent to the town of Soda Springs, Placer County, California. The Serene Lakes district maintains its own water treatment facility (disinfection by chlorination), which obtains its source water from the lakes that are within the district. The community is made up primarily of week-end or summer cottages so the population is mainly transient, residing there only on week-ends or holidays to take advantage of the nearby ski areas. Over 100 individuals showed symptoms of acute gastroenteritis (vomiting, diarrhea) after staying at different cabins or cottages within the district. The only common feature to the outbreak was the water supply. After appropriate epidemiological investigation had taken place to assure a suspect waterborne cause, samples of the water were obtained by our laboratory for viral assay. A 100 gallon sample was concentrated at the water treatment facility and samples were obtained from a cottage involved in the outbreak and from a fire hydrant on the water system. No viruses (<1 virus per 50 gallons of sample) were isolated from these samples. A later sample of source water before chlorination proved to have a virus concentration of 220 plaque forming units (pfu) of virus per gallon of water. These viruses were identified as reovirus type 2 and echovirus type 14.

Stool samples and samples of acute and convalescent serum were obtained from several of the individuals with gastroenteritis symptoms and were examined by immune electron microscopy. Positive reactions were obtained with several of the convalescent sera to a 27 nm parvovirus like particle in the stool specimens. Ten paired serum specimens were sent to Dr. Harry Greenberg at the National Institutes of Health for radio-immunoassay against Norwalk like agents. Eight of the 10 pairs of sera showed evidence of an $\geq$4 fold rise in titer to Norwalk virus. The Norwalk-like agent was then thought to be either partially or wholly responsible for the outbreak of gastroenteritis. The results, particularly the isolation of echovirus and reovirus strongly support the contention that human fecal material had contaminated Lake Serena.

GRINDSTONE INDIAN RESERVATION OUTBREAK

An outbreak of gastroenteritis occurred in May 1980 on the Grindstone Indian Reservation near Elk Creek, Glenn County, California. Drinking water was thought to be the source of the outbreak and the water virology unit of the VRDL was asked to assay the water for possible viral pathogens. Stool and acute and convalescent blood specimens were collected from as many of the ill individuals as possible.

The community water supply consists of an infiltration gallery with a wet well next to a creek. The water is chlorinated and subsequently filtered before going into the distribution system.

One hundred gallon samples of water were concentrated at two sites at the reservation; 1) source water before chlorination and 2) an external tap

at a residence. Results of these samples showed 0.8 pfu of virus per gallon in the source water and 9.6 pfu of virus per gallon at the residence. All of the isolated viruses were identified as coxsackie B3.

Results of immune electron microscopy showed a 27 nm parvovirus-like particle in a stool specimen which reacted with convalescent serum samples collected from six individuals experiencing gastroenteritis. These results indicate that a Norwalk-like virus agent was probably the cause of the outbreak. The coxsackie B3 virus probably did not contribute to the gastroenteritis, but isolation of this agent proves human fecal contamination of the water supply. A faulty chlorinator in the water treatment system resulted in unchlorinated water being distributed within the system. Subsequent concentrating and assaying for viruses after the chlorinator was repaired resulted in no viruses being isolated.

AUBURN GASTROENTERITIS OUTBREAK

In March of this year an outbreak of acute gastroenteritis occured in Auburn, Placer County, California, among two first-grade classes and associated adults (approximately 60 individuals) who had gone on a joint field trip. An orange juice drink which had been prepared with water from a private well was implicated as the common source of the outbreak by epidemiological investigation.

Samples of well water were concentrated for virus assay and stool samples and acute and convalescent serum samples were requested from the individuals involved. No viruses were isolated from the concentrated well water samples nor were any virus-like particles seen by IEM in the stool samples.

SQUAW VALLEY GASTROENTERITIS OUTBREAK

In April of 1981 an outbreak of acute gastroenteritis occurred among individuals who had eaten or drank at the Gold Coast restaurant in the Squaw Valley ski area, Placer County, California. Preliminary investigation suggested that the outbreak was waterborne and the water virology laboratory was requested to assay the water at the restaurant for viruses.

The restaurant is located at approximately the 8,000 foot level with access to the area for skiing by chair-lift or gondola. Equipment and personnel for virus concentration was transported to the site by Sno-cat. Source water supplied to the restaurant was obtained from a small reservoir which remained frozen over for most of the ski season. Approximately 150 gallons of water, obtained from a tap in the kitchen of the restaurant, was concentrated using the membrane filter technique. After elution and further concentration in the laboratory the concentrated samples were inoculated onto RD, BGMK, HFDK, and MFK cell cultures. No viruses were isolated. The RD and MFK cell systems were blind passaged. Again no viruses were isolated.

Requests were made for acute and convalescent serum specimens and for stool specimens from the infected individuals. No samples were obtained of acute serum nor of stool samples. Several convalescent serum samples were obtained, however, which were tested against the Grindstone parvovirus-like agent with negative results. Portions of the convalescent serum samples were shipped to the CDC for testing by RIA for antibody to the Norwalk agent. None of the samples submitted were positive for this agent.

Water samples from the supplies implicated in the gastroenteritis outbreaks were collected at varying times subsequent to the outbreaks. Therefore, the microbiological analysis does not necessarily reflect water quality at the time of the outbreak. However, an indication of general water quality can be determined from the coliform analysis performed on grab samples taken during the collection of large-volume virus samples. In addition to coliform determinations, fecal streptococcal analysis of outbreak water samples was initiated in June 1980.

A summary of the microbiological data is presented in Table 4. As can be seen, coliforms were isolated each time viruses were recovered. However, the coliform counts were relatively low in the untreated Soda Springs sample that yielded reovirus 2 and echovirus 14. No viruses were isolated from water supplies that would be considered potable based on coliform levels or chlorination practice. Fecal streptoccus findings tended to parallel the coliform recovery pattern.

ISOLATION OF GASTROENTERITIS AGENTS

Cell cultures from primates (chimpanzee, marmoset, monkey) were used in attempts to culture the agents producing gastroenteritis in humans (parvovirus-like particles, rotaviruses). Such techniques as centrifugation of inoculum onto cells and treatment of cells with dilute solutions of trypsin were used separately and in combination. Evidence of growth was to be determined by using convalescent phase human sera from individuals with gastroenteritis which were positive by IEM.

In no case was a virus isolated nor growth detected by these techniques.

DISCUSSION

Although it has been reported in the literature that an average of 50% of seeded virus added to treated sewage effluent can be recovered using pleated membrane filters (Gerba et al.)*, the results of this study would tend to indicate either that wild viruses cannot be recovered to the extent described or that viruses were not present in the disinfected final effluent of the two treatment plants. The poor quality of the effluent especially at the San Pablo plant, when the turbidity, suspended solids, and BOD were high, together with a high coliform count would lead one to expect to recover viruses. On only two occasions were viruses isolated, however, and in low numbers. Possibly the organic load of the effluent water competes with viruses for adsorption sites on the pleated membrane filters when this technique of concentration is used. The actual viral content of a typical disinfected secondary effluent therefore could probably not be ascertained from the study because of the shortcomings of the virus recovery method.

The recovery of viruses from the influent and from the pre-disinfected final effluent showed that on the average up to a 3 $\log_{10}$ reduction of viruses occurred during the treatment process. Depending upon the cell type used for viral isolation, a late summer peak of the number of viruses isolated occurred only during the first year of the study.

Identification of the isolated viruses showed that there was no difference in the types of viruses recovered at different points along the treatment process. Because the number of viruses recovered in the influent was so large, only representative isolates were identified while all of the viruses isolated from the pre-disinfected final effluent were identified. Reoviruses were the most frequently isolated during the course of the study with echovirus type 7 being the second most frequently isolated.

If it evident from the number and kinds of viruses recovered in the cell culture systems used in this study that any type of proposed regulations concerning viruses in water (i.e. one virus per gallon, one virus per 100 gallons), will have to be qualified as to the kind of virus allowed. This is due to the variation in the sensitivities of different cell culture systems to different viruses. Table 3 exemplifies this variation in sensitivity of different cell culture systems to group A coxsackieviruses.

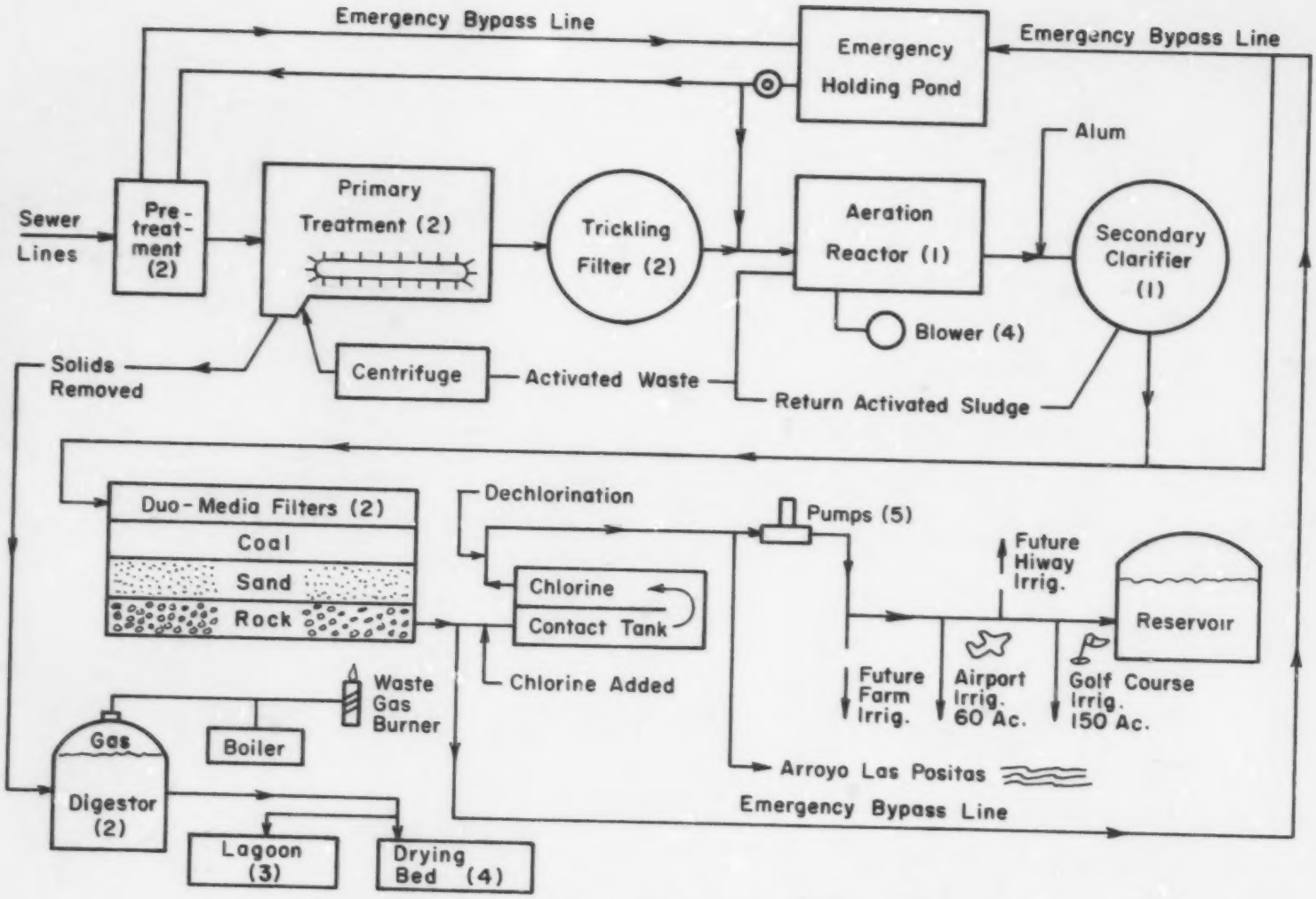
*Gerba, C. P., S. R. Farrah, S. M. Goyal, C. Wallis, and J. L. Melnick. Concentration of Enteroviruses from Large Volumes of Tap Water, Treated Sewage, and Seawater. Appl. & Environ. Microbiol. 35: 540-548, 1978.

It has been reported (Schmidt, et al. 1975)*, that RD cells are somewhat more sensitive to these viruses than other conventional cell culture systems. These results were confirmed in these studies by the greater number of isolations of group A coxsackieviruses in RD cells (117) as compared to the number of isolates in the three other cell systems combined (2a).

In three of the five waterborne outbreaks of gastroenteritis investigated, human viruses were isolated from the water, indicating that the water supply was contaminated with human fecal material. In each of these three outbreaks a parvovirus-like agent was implicated as the causative agent by immune electron microscopy of stools collected from individuals involved in the gastroenteritis outbreaks.

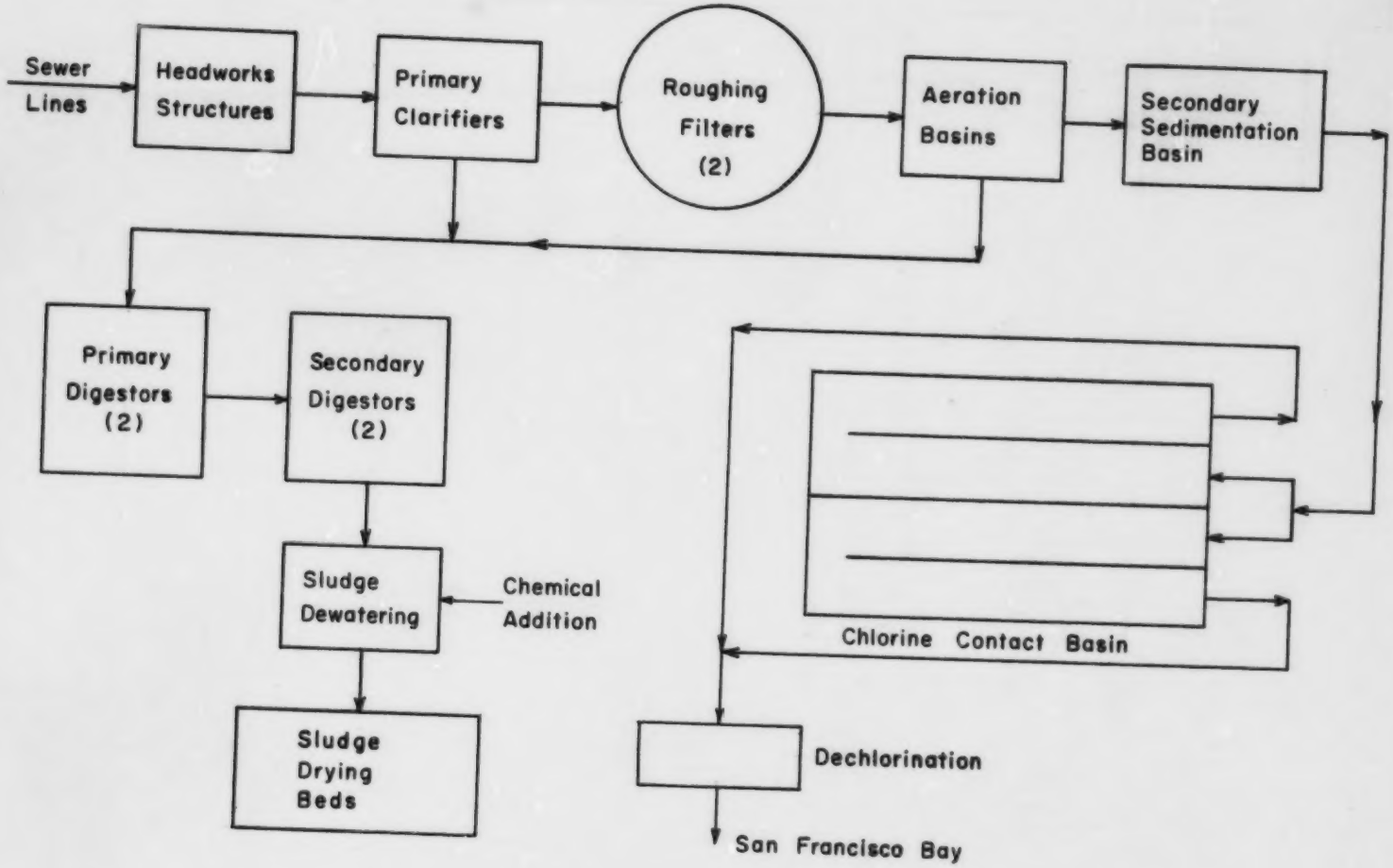
Although efforts to grow the at-present-uncultivable agents (parvovirus, rotavirus, hepatitis virus) were not successful, the time and effort expended on this phase of the work was not extensive enough to detail or discuss thoroughly.

*Schmidt, N. J., H. H. Ho, and E. H. Lennette. Propagation and Isolation of Group A Coxsackieviruses in PD Cells. J. Clin. Microbiol. 2: 183-185, 1975.



LIVERMORE WATER RECLAMATION PLANT

SCHEMATIC FLOW DIAGRAM
5 MILLION GALLON PER DAY AVERAGE DRY WEATHER



The San Pablo Sanitary District
Water Pollution Control Plant

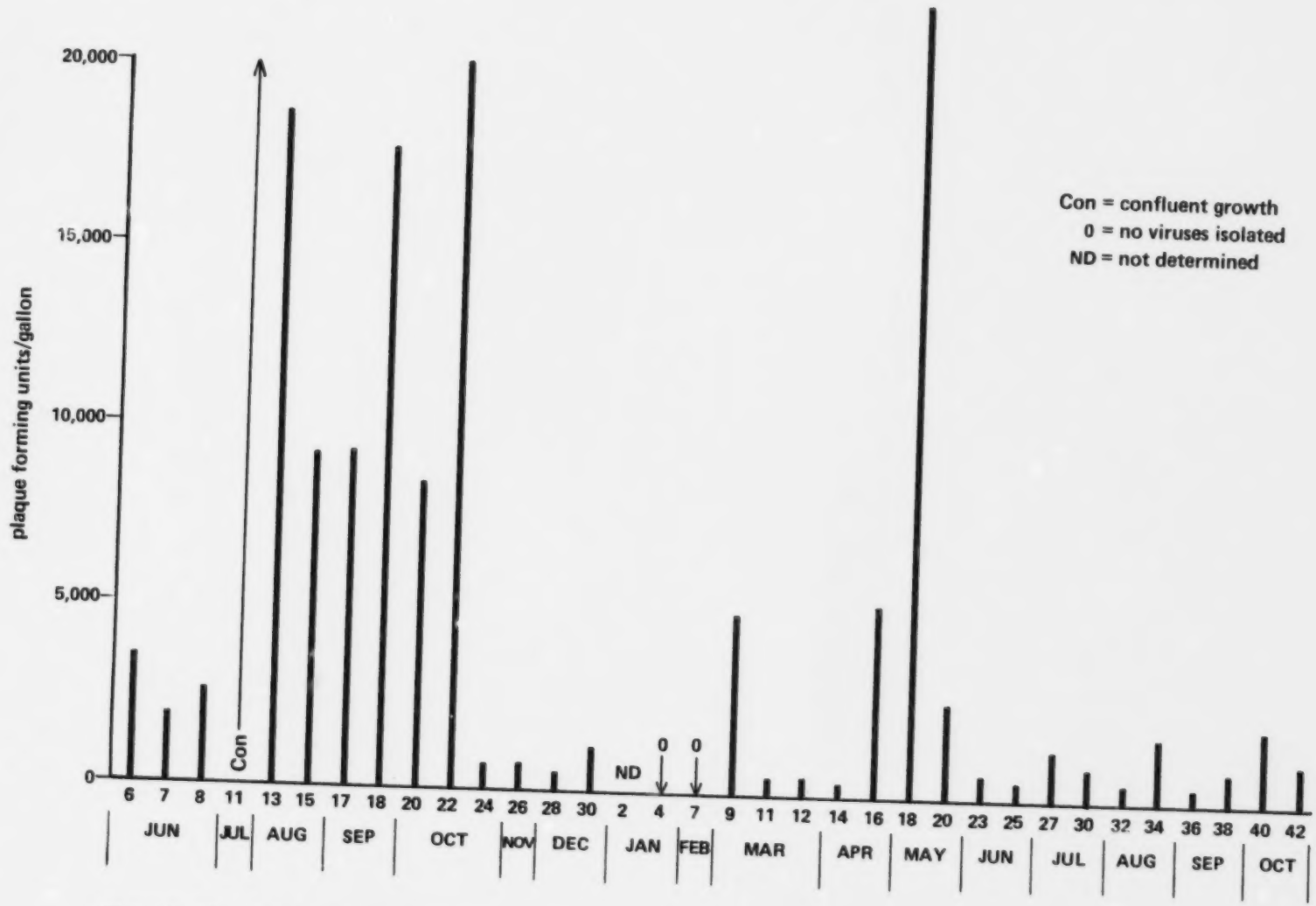


Figure 3. Virus isolation in Buffalo green monkey kidney cells. Influent, San Pablo treatment plant, June 1979 - October 1980.

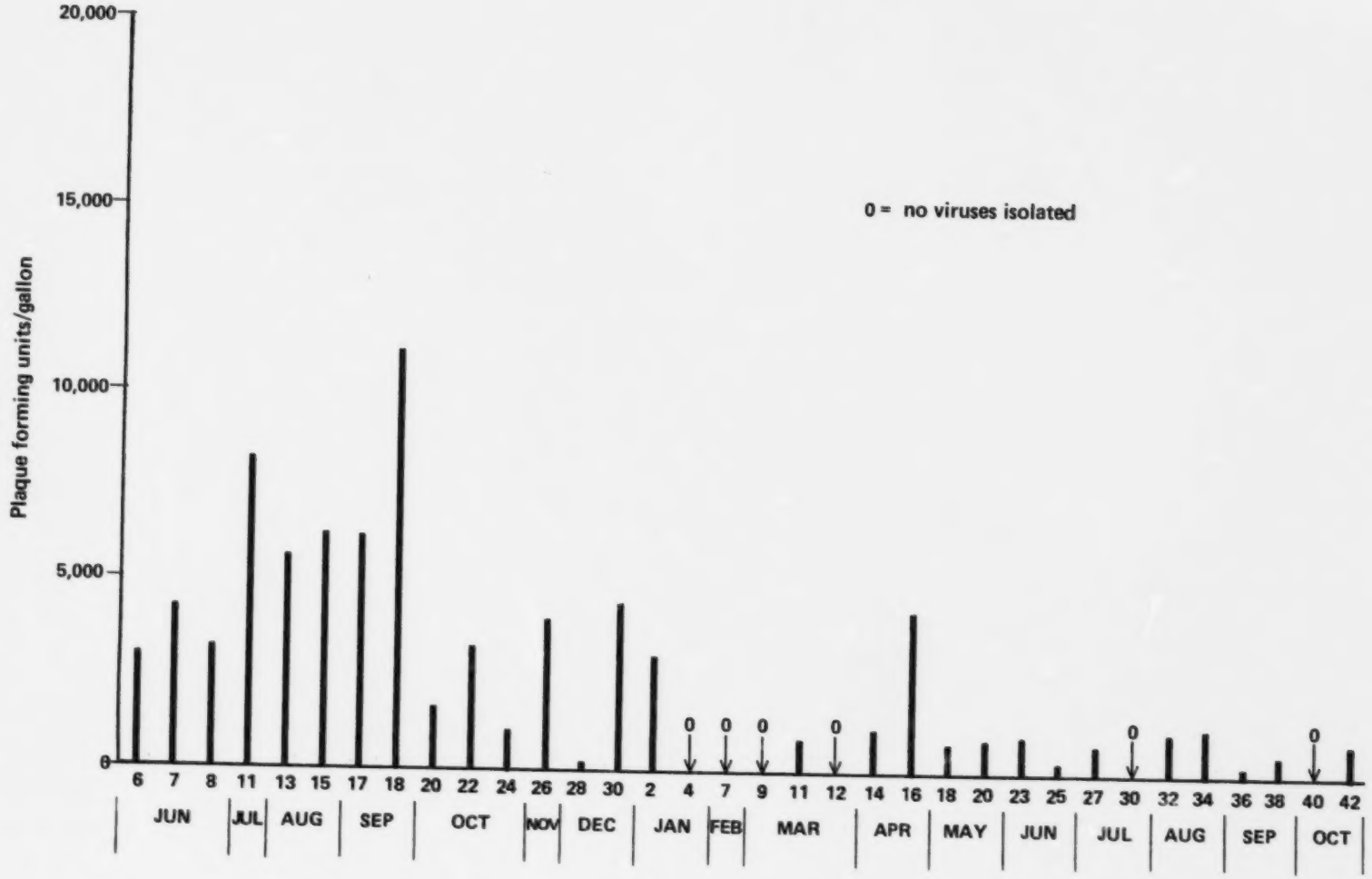

20,000
15,000
10,000
5,000
0
Plaque forming units/gallon
0 = no viruses isolated
6 7 8 11 13 15 17 18 20 22 24 26 28 30 2 4 7 9 11 12 14 16 18 20 23 25 27 30 32 34 36 38 40 42
JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT

Figure 4. Virus isolation in human rhabdomyosarcoma cells (RD). Influent, San Pablo treatment plant, June 1979 - October 1980.

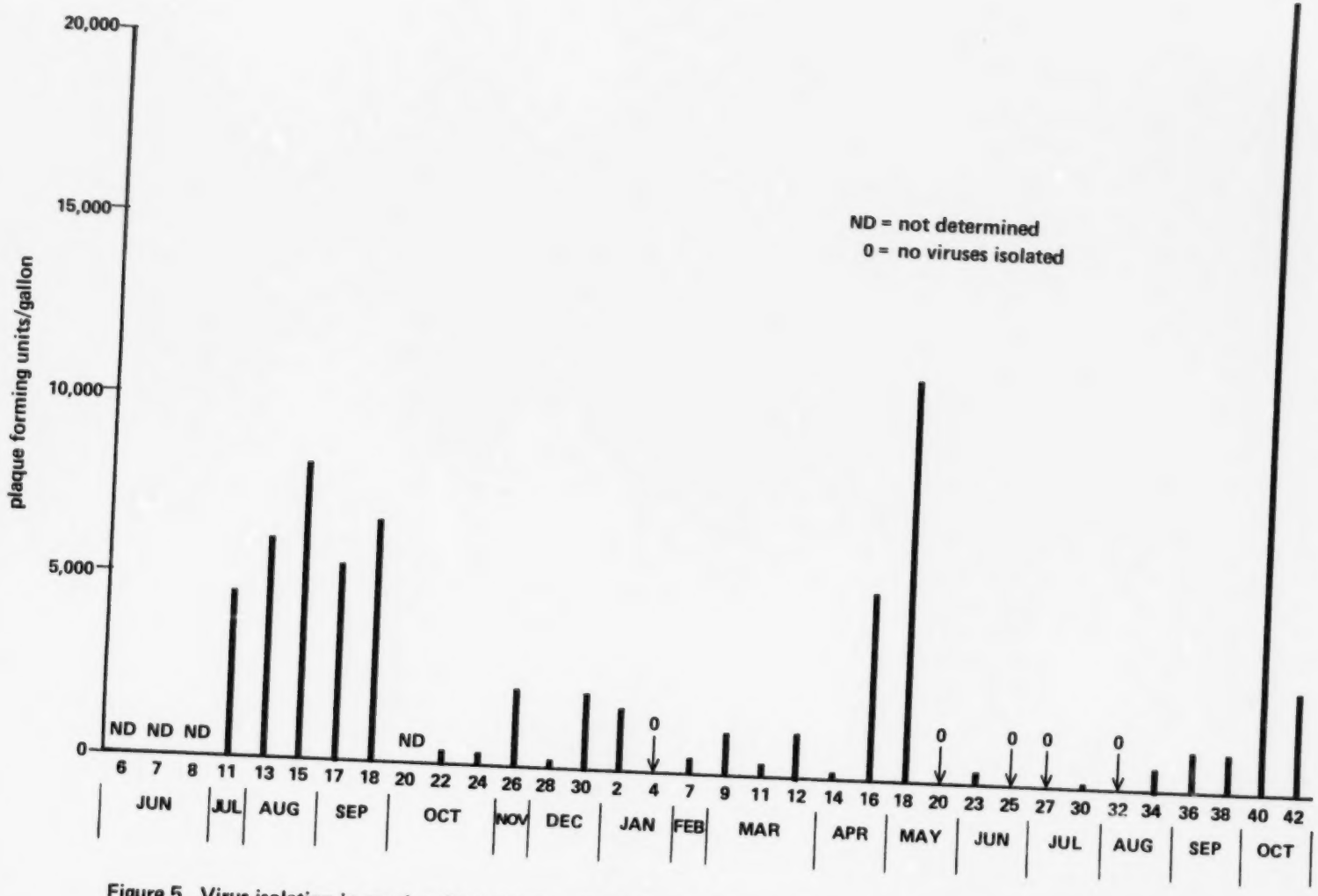


Figure 5. Virus isolation in monkey fetal kidney cells. Influent, San Pablo treatment plant, June 1979 - October 1980.

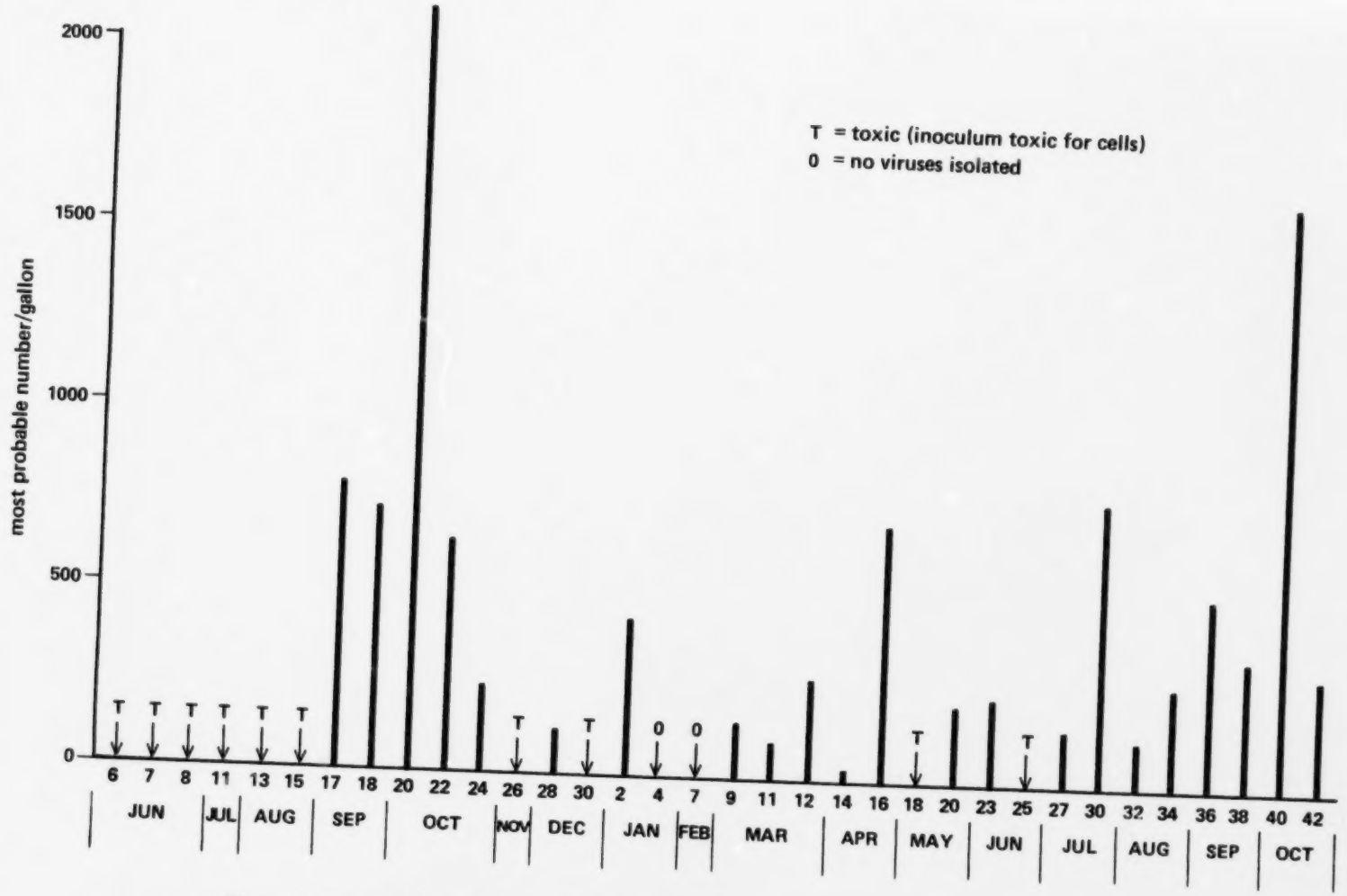

T = toxic (inoculum toxic for cells)
0 = no viruses isolated
most probable number/gallon
2000
1500
1000
500
0
T T T T T T T T T T T 0 0 T T
6 7 8 11 13 15 17 18 20 22 24 26 28 30 2 4 7 9 11 12 14 16 18 20 23 25 27 30 32 34 36 38 40 42
JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT

Figure 6. Virus isolation in human fetal kidney cells. Influent, San Pablo treatment plant, June 1979 - October 1980.

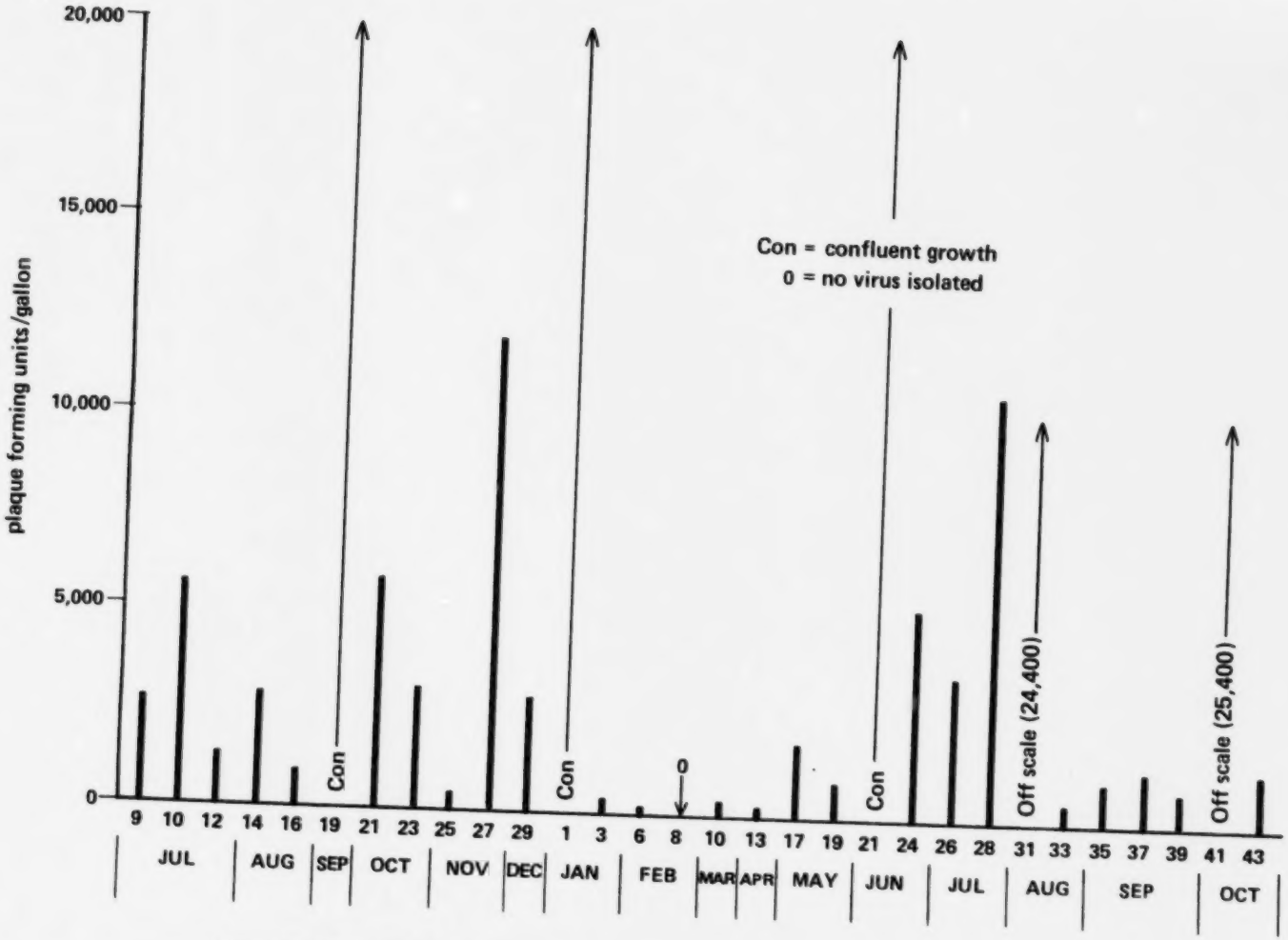


Figure 7. Virus isolation in Buffalo green monkey cells. Influent, Livermore treatment plant, July 1979 - October 1980.

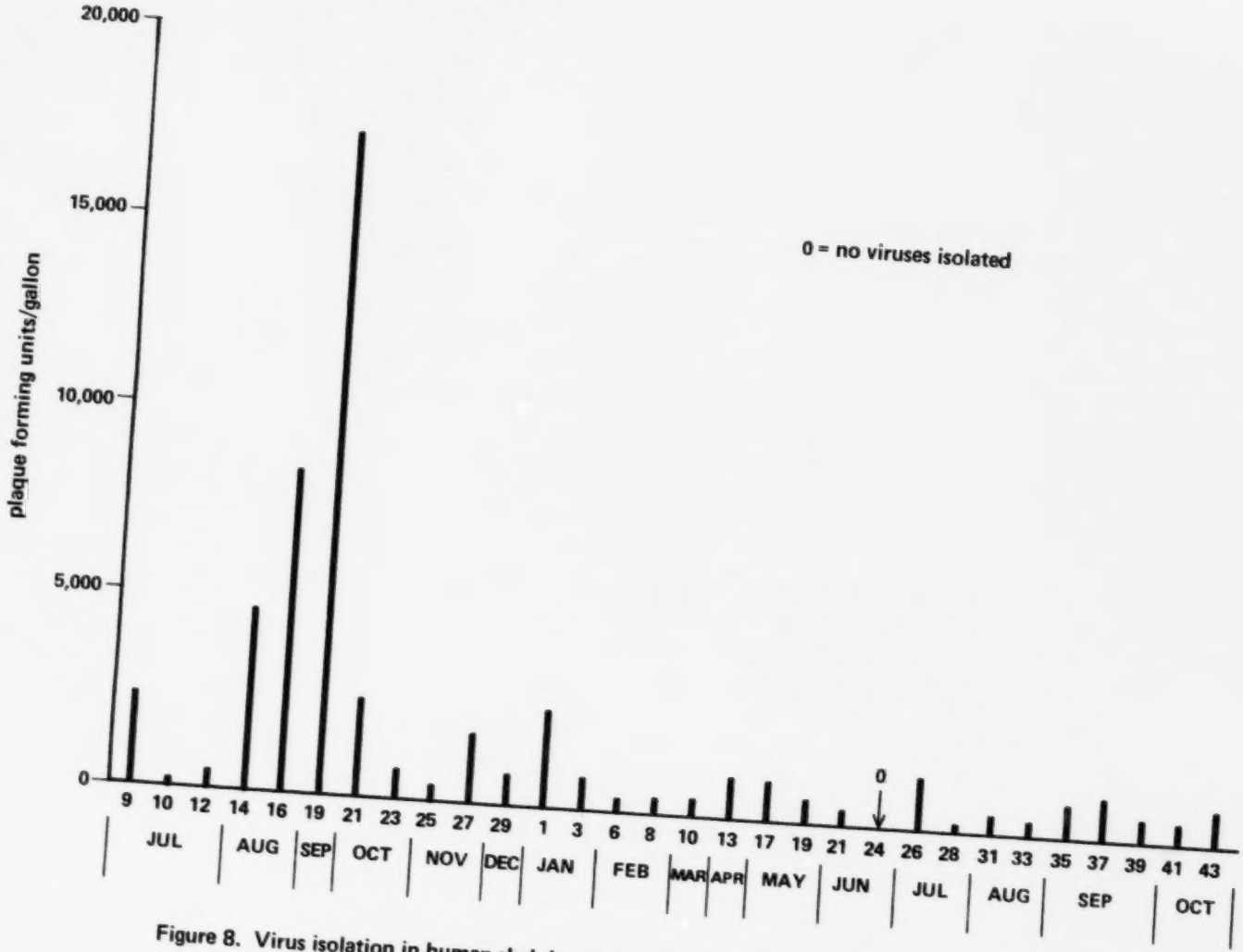


Figure 8. Virus isolation in human rhabdomyosarcoma cells. Influent, Livermore treatment plant, July 1979 - October 1980.

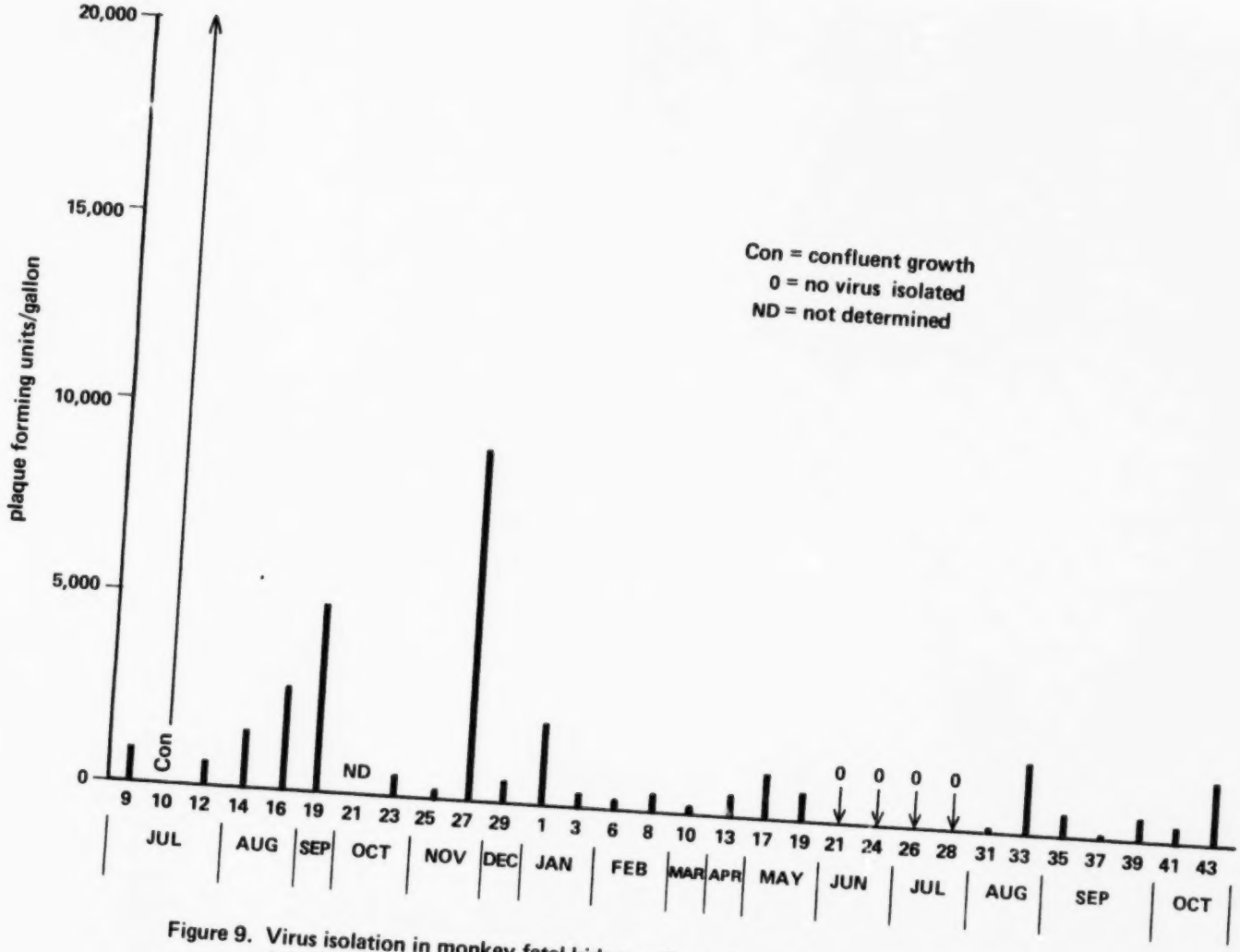

20,000
15,000
10,000
5,000
0
plaque forming units/gallon
Con = confluent growth
0 = no virus isolated
ND = not determined
Con
ND
0 0 0 0
9 10 12 14 16 19 21 23 25 27 29 1 3 6 8 10 13 17 19 21 24 26 28 31 33 35 37 39 41 43
JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT

Figure 9. Virus isolation in monkey fetal kidney cells. Influent, Livermore treatment plant, July 1979 -October 1980.

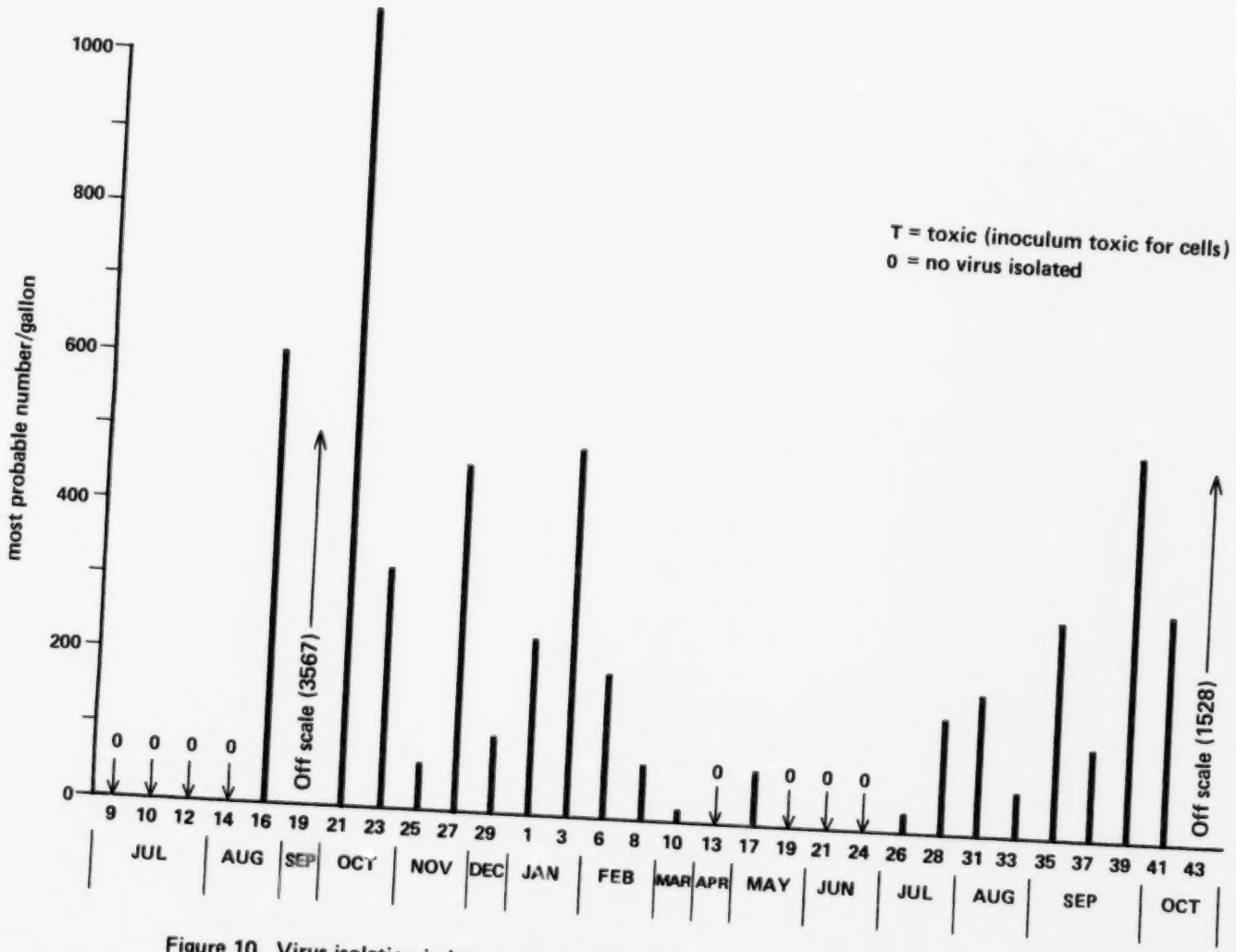


Figure 10. Virus isolation in human fetal kidney cells. Influent, Livermore treatment plant, July 1979 - October 1980.

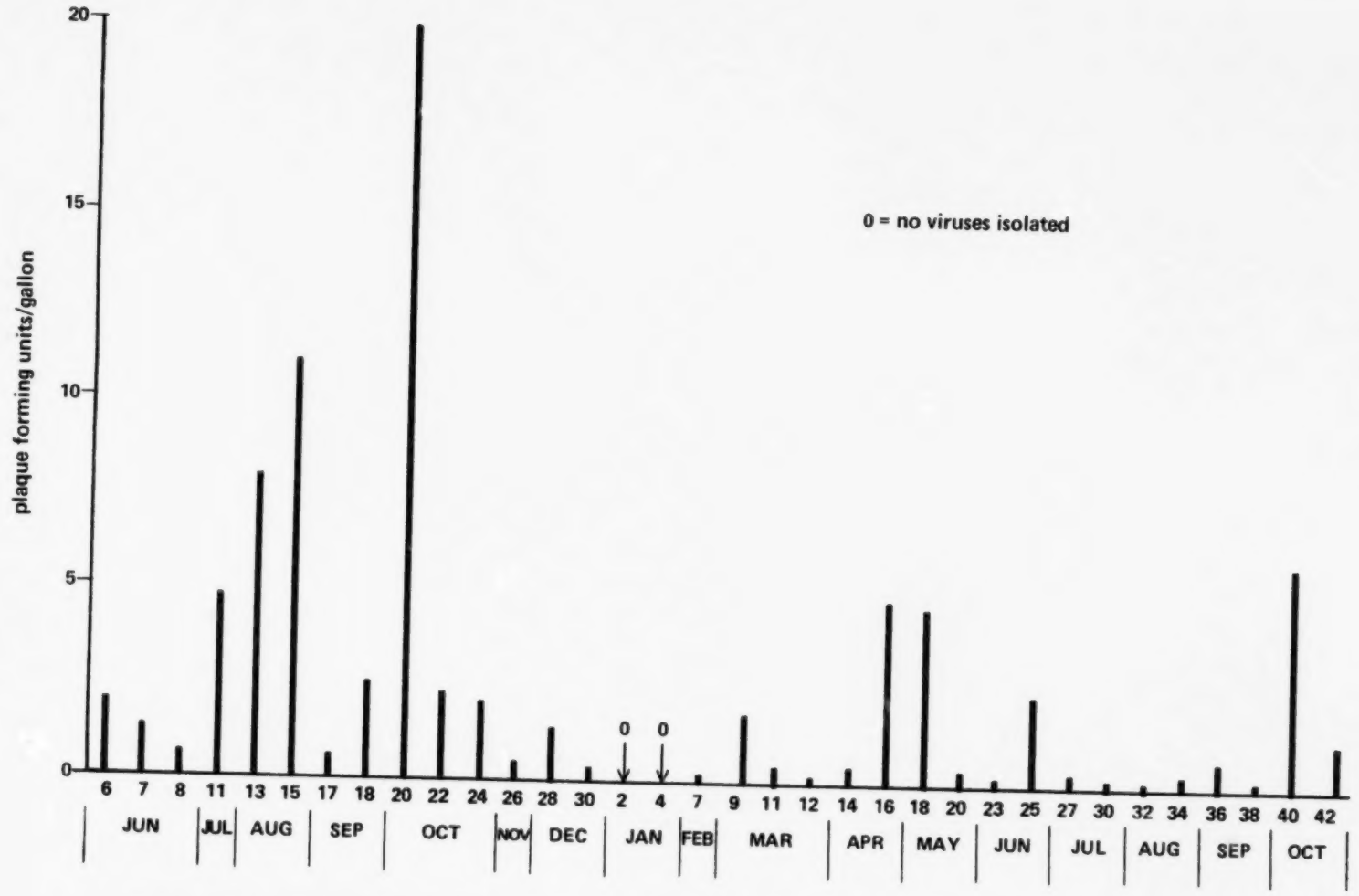


Figure 11. Virus isolation in Buffalo green monkey cells. Effluent (pre-chlorination), San Pablo treatment plant, June 1979 - October 1980.

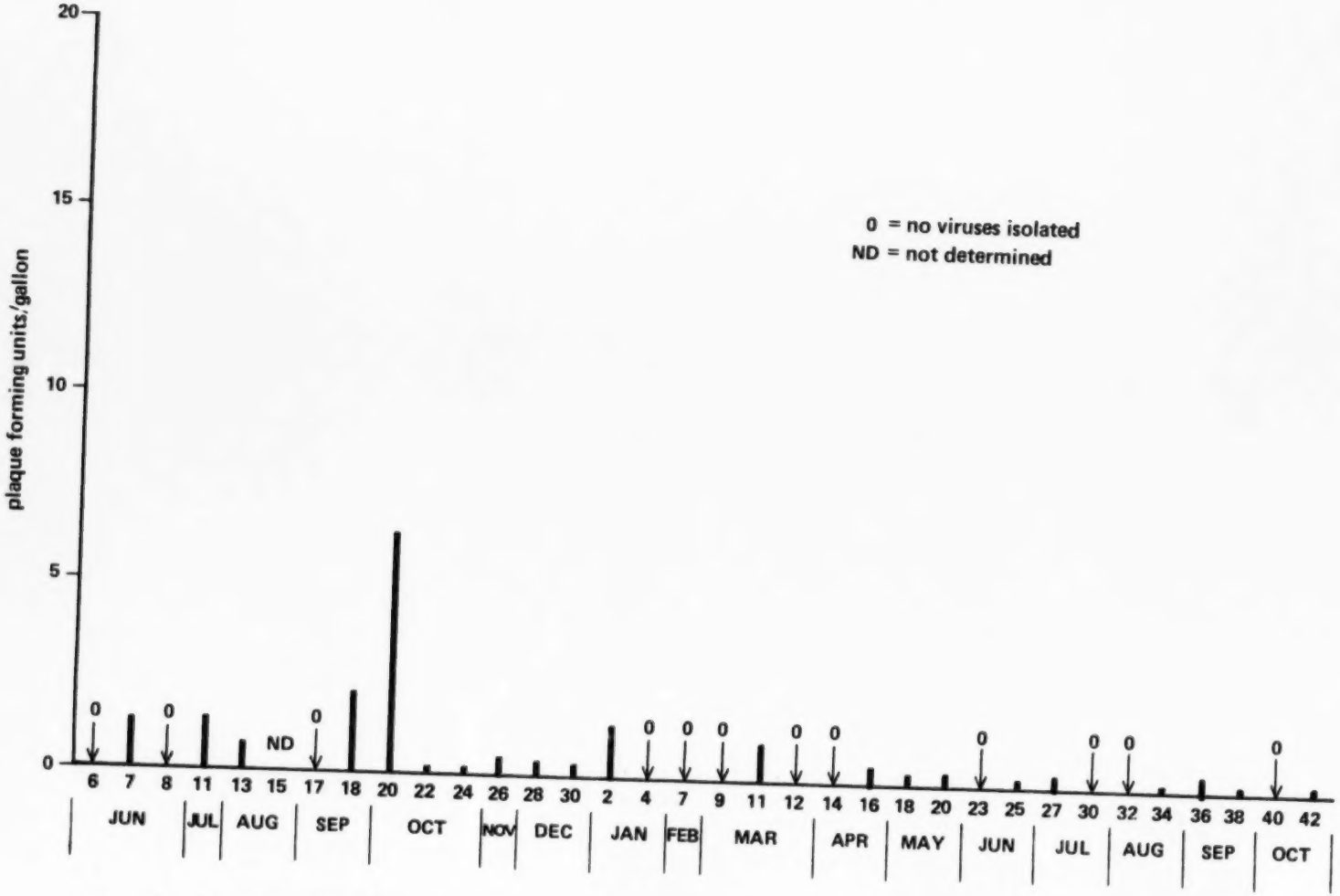

20
15
10
5
0
plaque forming units/gallon
0 = no viruses isolated
ND = not determined
0 0 ND 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
6 7 8 11 13 15 17 18 20 22 24 26 28 30 2 4 7 9 11 12 14 16 18 20 23 25 27 30 32 34 36 38 40 42
JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT

Figure 12. Virus isolation in human rhabdomyosarcoma cells. Effluent (pre-chlorination), San Pablo treatment plant, June 1979 - October 1980.

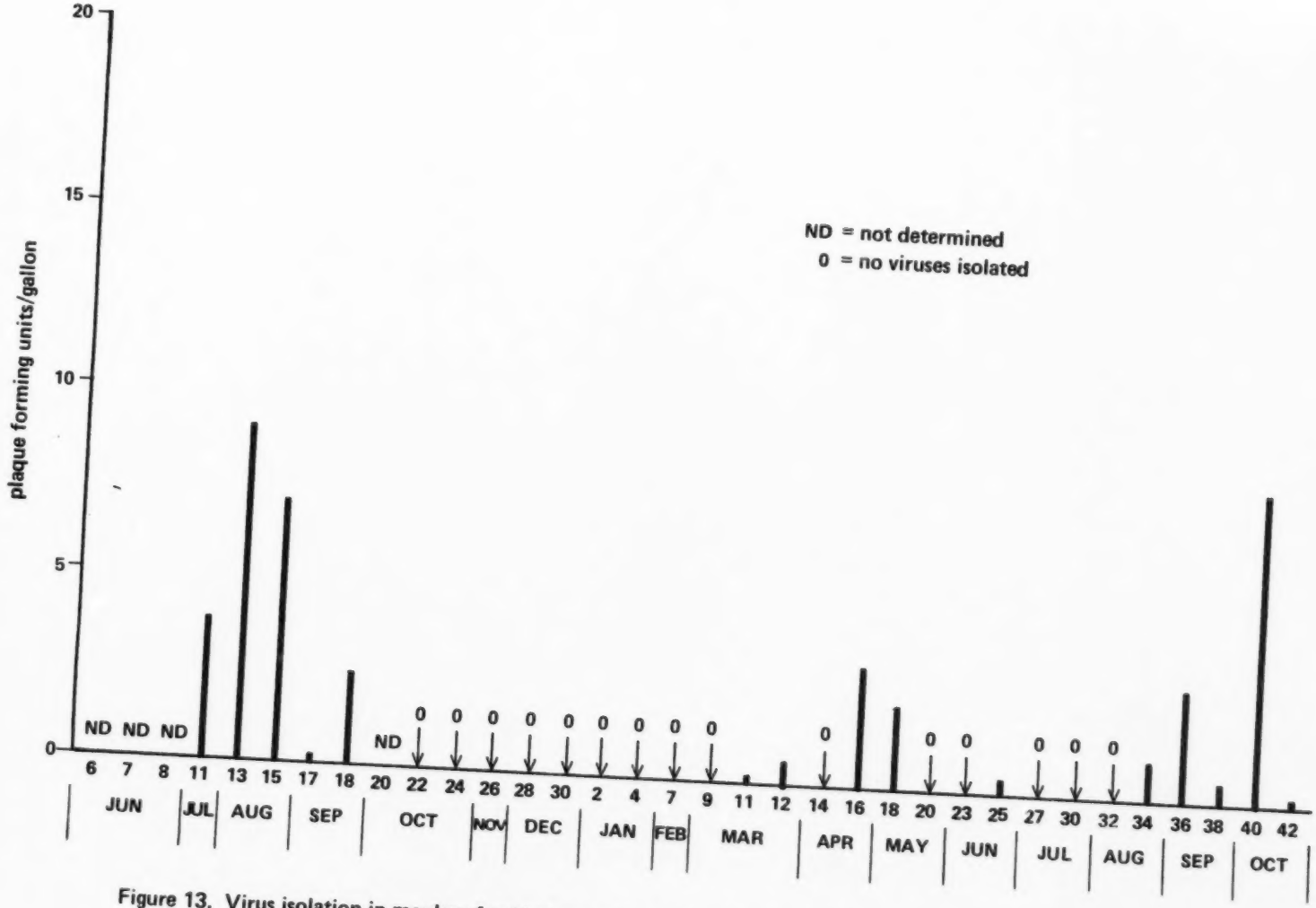


Figure 13. Virus isolation in monkey fetal kidney cells. Effluent (pre-chlorination), San Pablo treatment plant, June 1979 - October 1980.

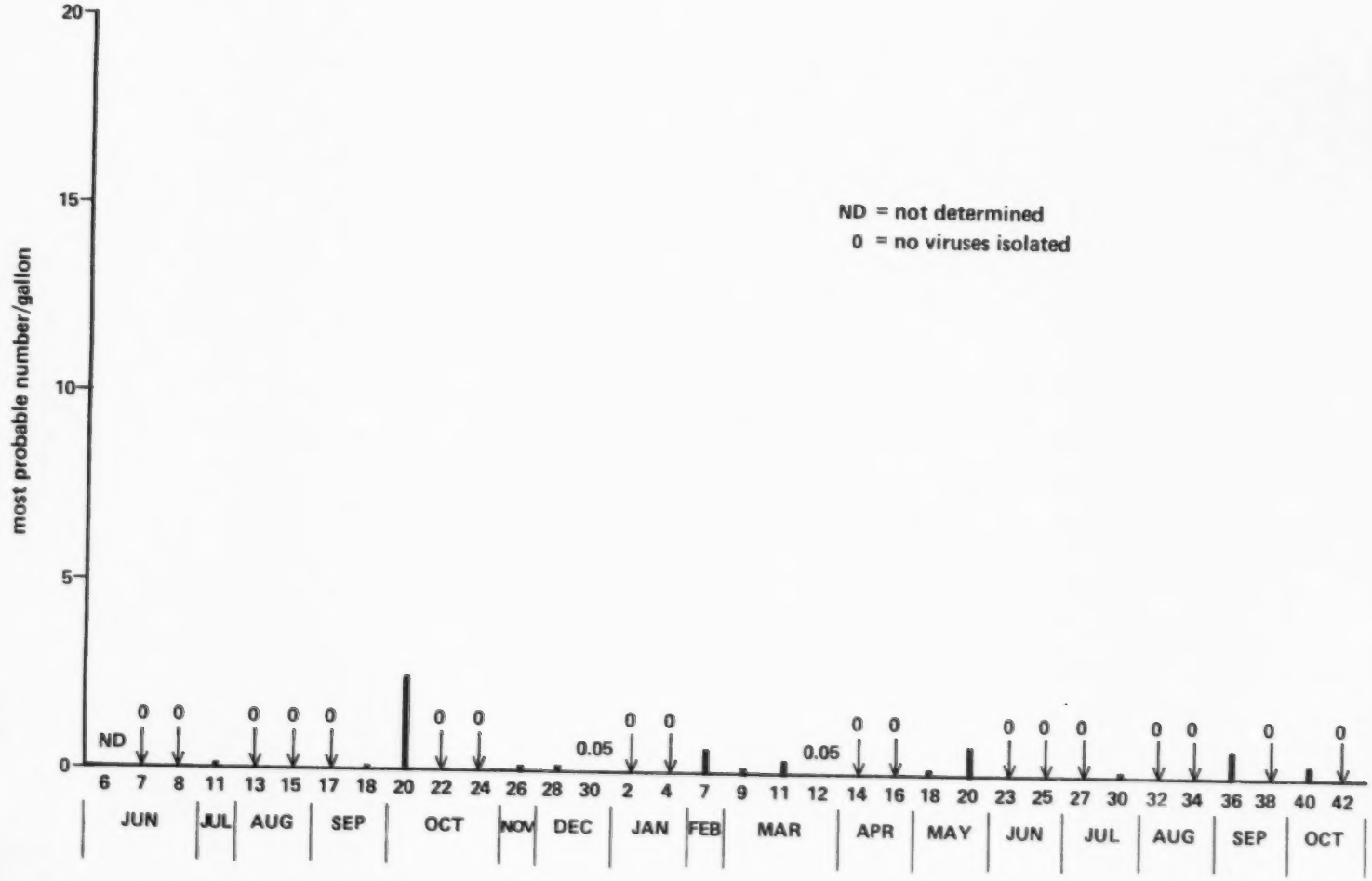


Figure 14. Virus isolation in human fetal kidney cells. Effluent (pre-chlorination), San Pablo treatment plant, June 1979 - October 1980.

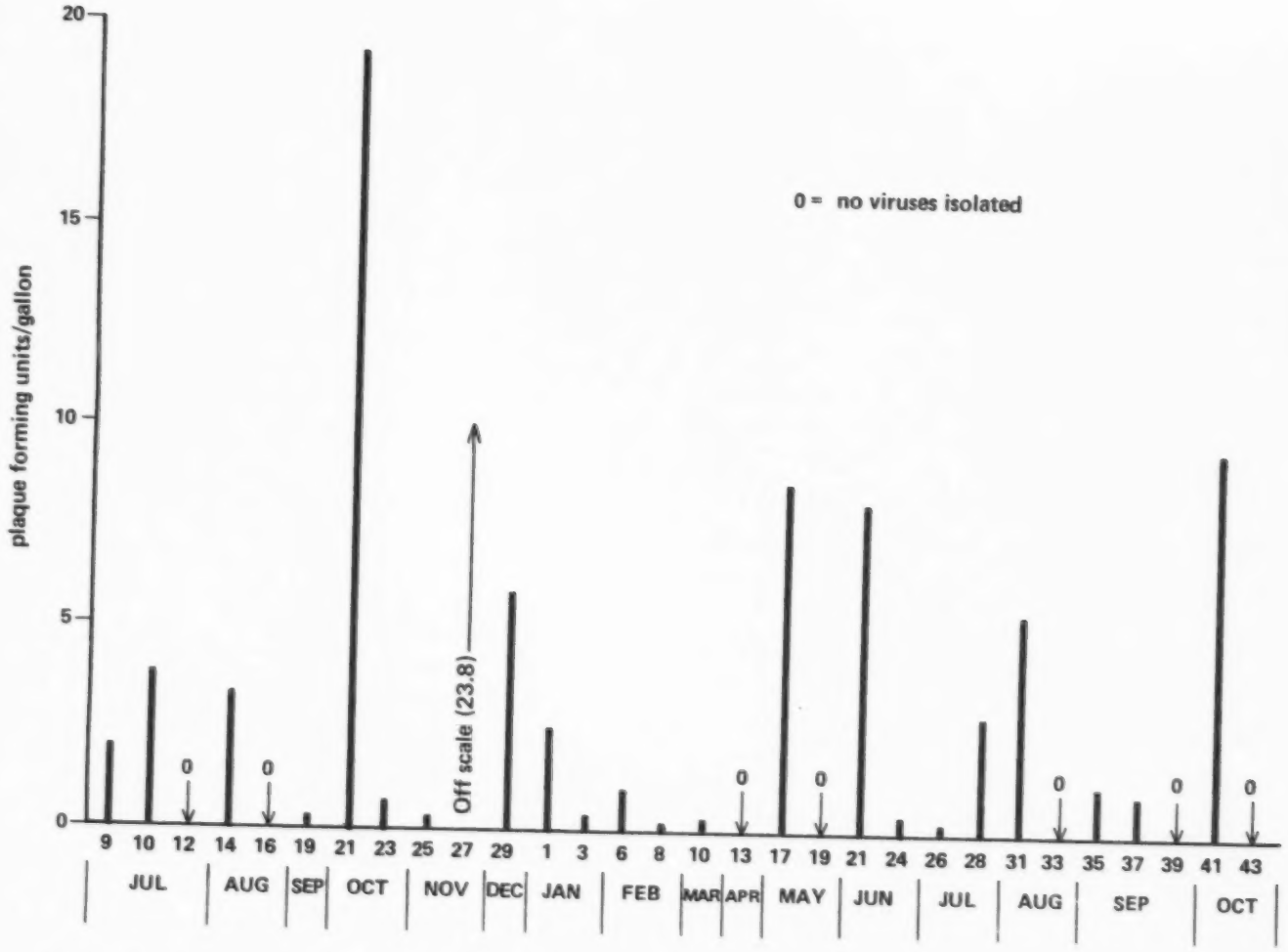


Figure 15. Virus isolation in Buffalo green monkey cells. Effluent (pre-chlorination), Livermore treatment plant, July 1979 - October 1980.

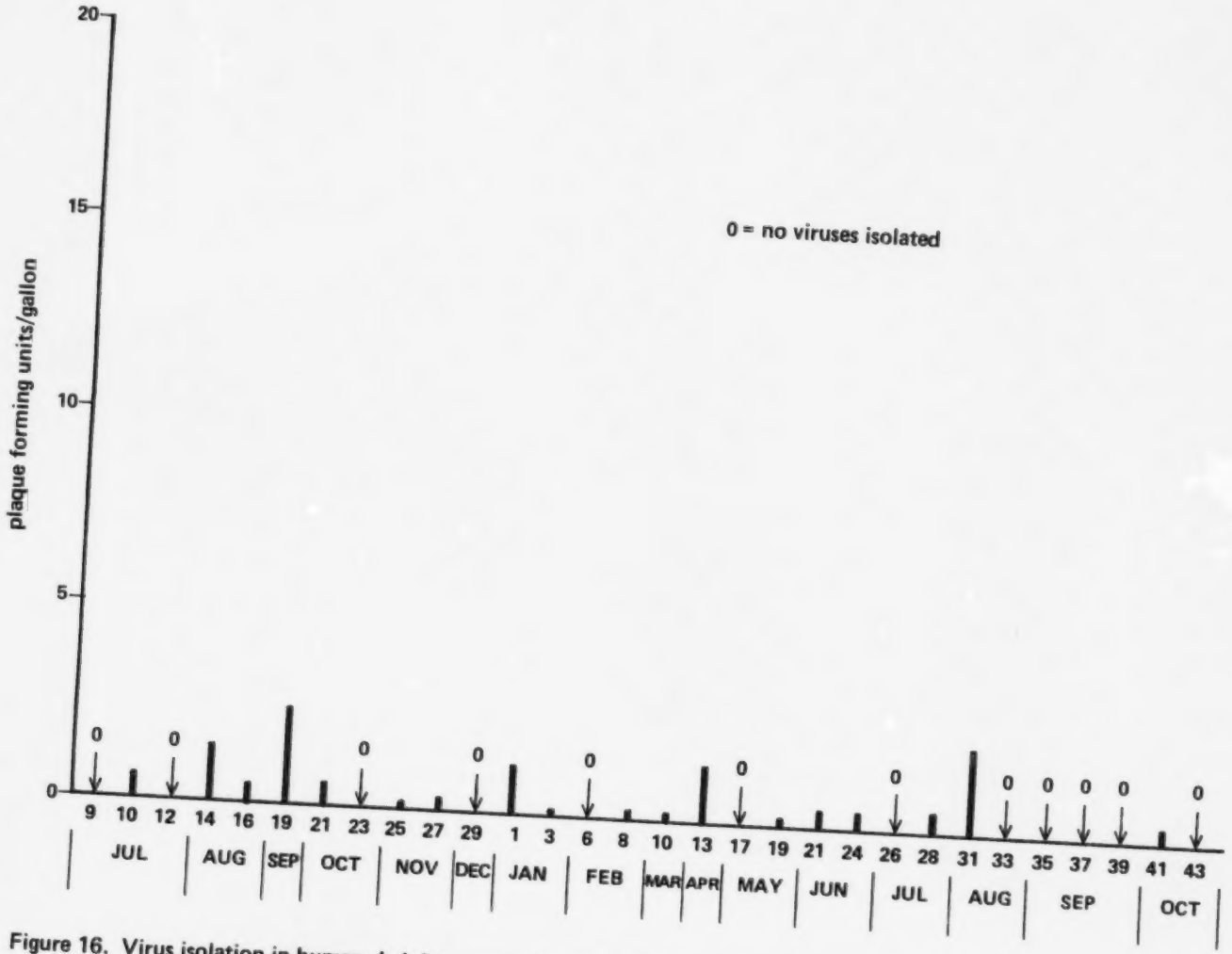


Figure 16. Virus isolation in human rhabdomyosarcoma cells. Effluent (pre-chlorination), Livermore treatment plant, July 1979 - October 1980.

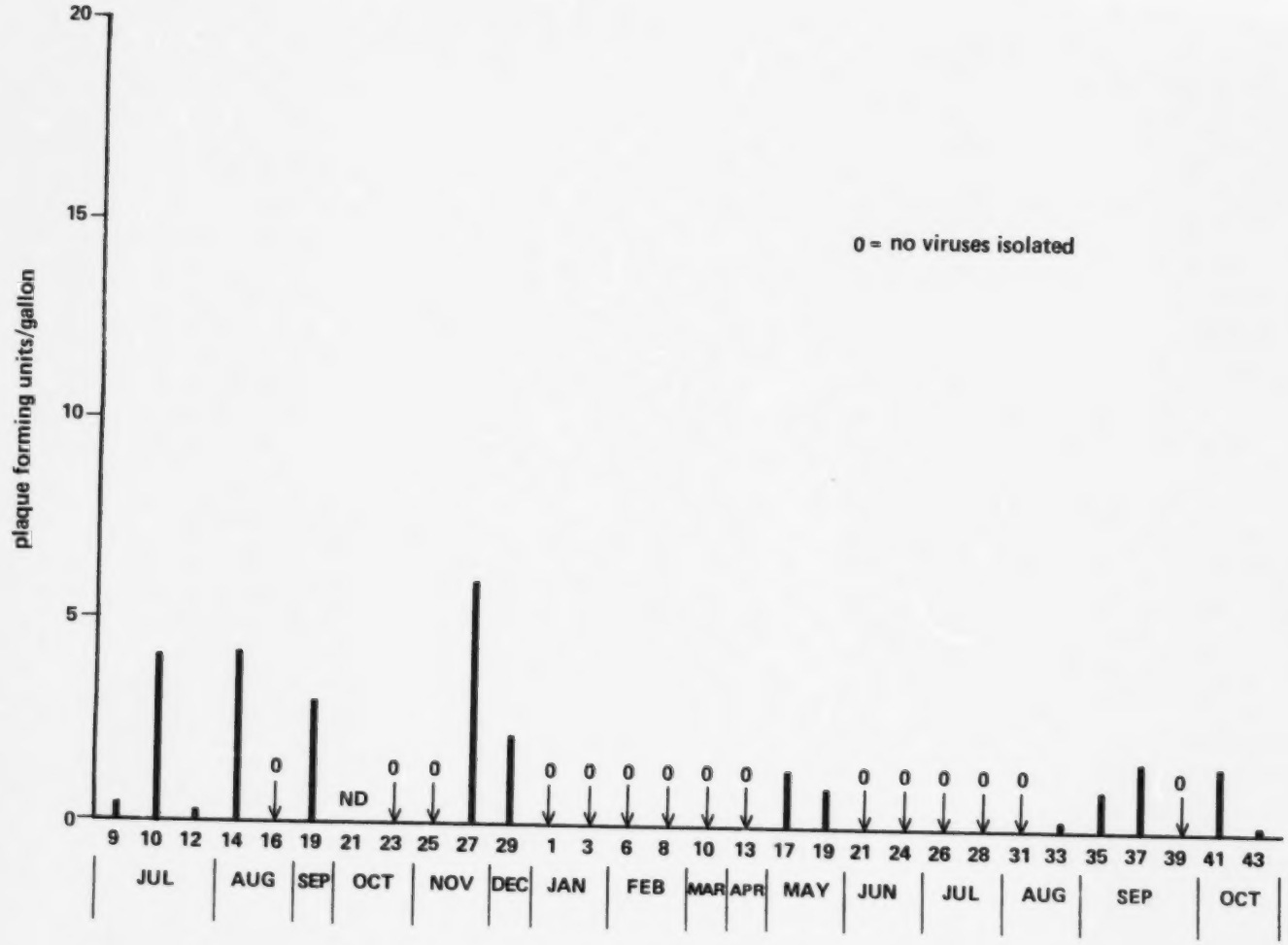


Figure 17. Virus isolation in monkey fetal kidney cells. Effluent (pre-chlorination), Livermore treatment plant, July 1979 - October 1980.

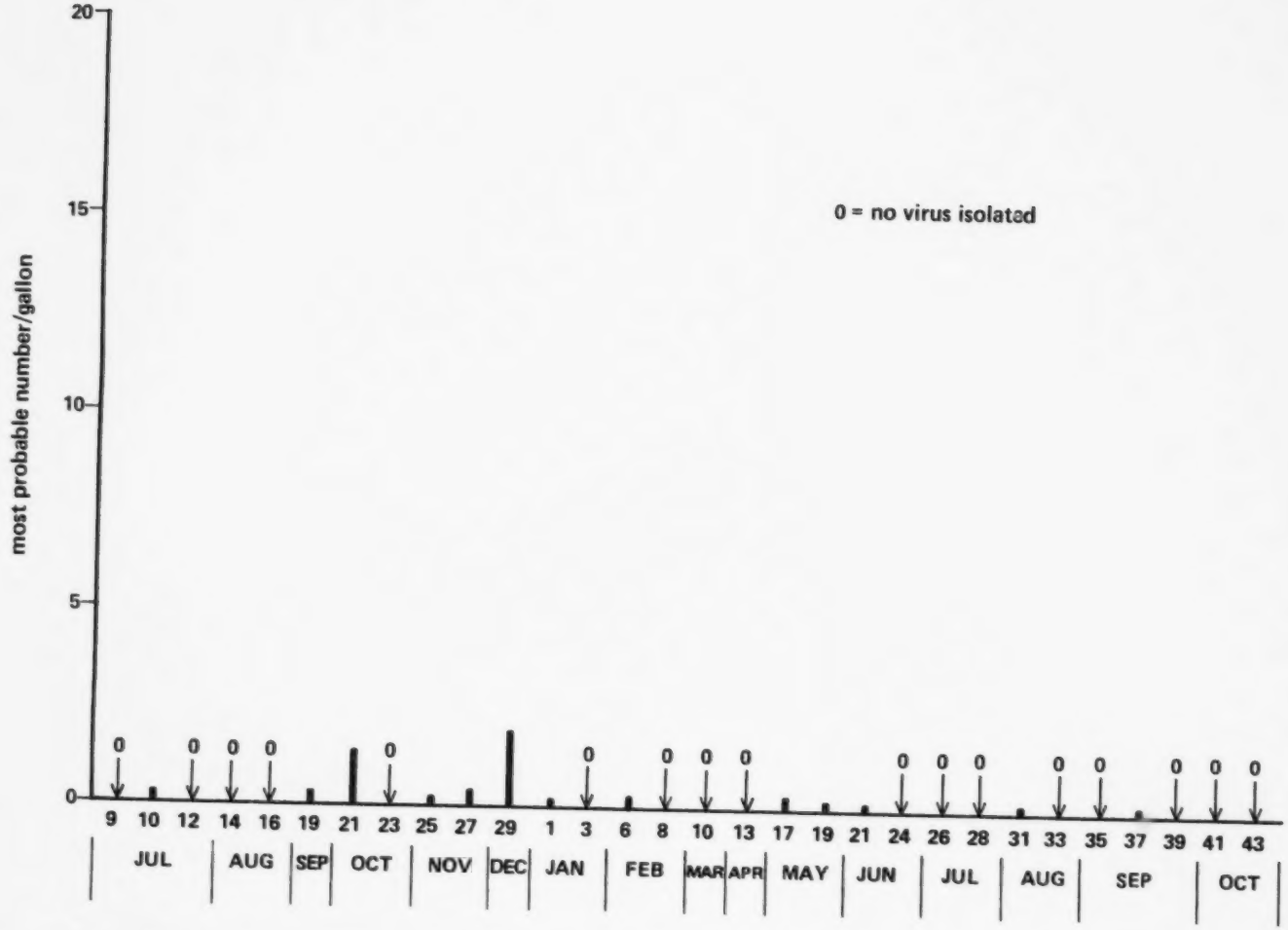


Figure 18. Virus isolation in human fetal kidney cells. Effluent (pre-chlorination), Livermore treatment plant, July 1979 - October 1980.

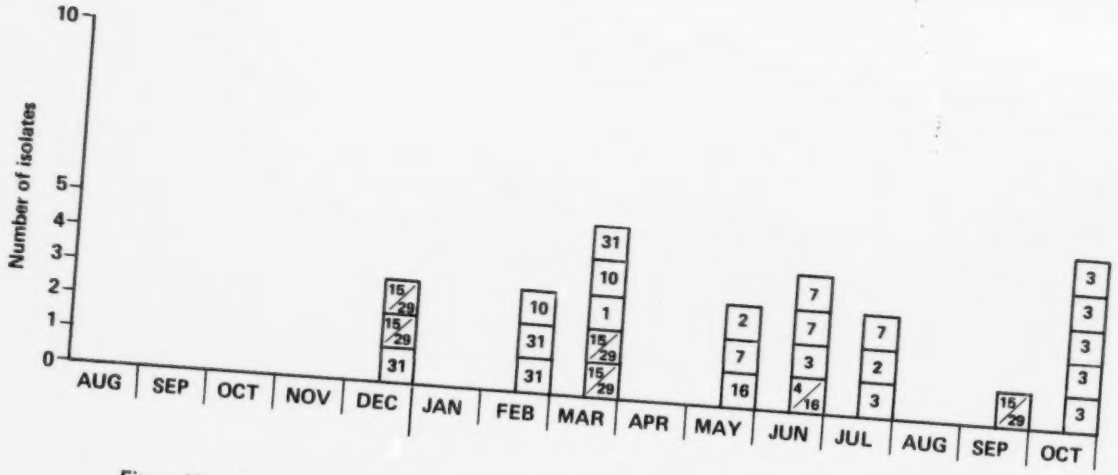


Figure 19. Adenoviruses isolated from San Pablo treatment plant, August 1979 – October 1980. Serotypes are indicated on the bar.

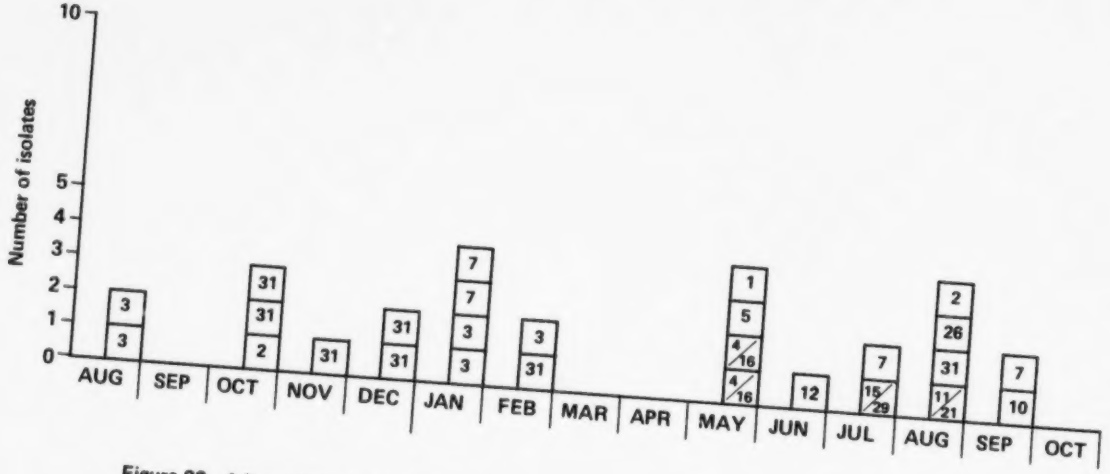


Figure 20. Adenoviruses isolated from Livermore treatment plant, August 1979 – October 1980 Serotypes are indicated on the bar.

TABLE 1. EFFLUENT WATER QUALITY DATA, SAN PABLO (SP) AND LIVERMORE (L) WASTEWATER TREATMENT PLANTS

Site	Flow rate (MGD)	Cl_2 residual (PPM)	Cl_2 contact time (min)	BOD (mg/1)	Turbidity (TU)	Suspended solids (mg/1)	Coliforms (total/fecal)	Fecal strep
SP	5.0	ND*	65	4.0	4.0	3.0	3500/20	--
SP	4.0	5.5	81	2.0	4.0	4.0	≥24,000/33	--
L	6.0	8.0	58	3.4	2.2	4.9	230/ND	--
L	5.5	11.0	63	2.2	0.8	0.8	5/<2	--
SP	6.1	5.5	59	24.0	45.0	65.0	≥24,000/16,000	--
L	5.5	12.0	63	0.5	0.6	2.2	11/<2	--
SP	5.5	4.2	72	15.0	38.0	67.0	≥24,000/24,000	--
L	6.0	19.0	58	8.2	3.7	7.0	20/<20	--
SP	7.5	5.5	34	6.0	7.0	5.0	2300/330	--
L	6.0	13.0	58	1.1	0.66	0.8	2/<2	--
SP	7.5	5.5	43	37.0	8.0	8.0	1700/17	--
SP	7.5	4.5	43	61.0	10.0	9.0	13,000/1200	--
L	6.0	14.0	58	2.3	1.1	0.7	13/<2	--
SP	8.5	4.5	39	27.0	12.0	11.0	24,000/140	--
L	5.5	14.0	63	2.6	0.7	0.3	46/5	--
SP	6.0	4.5	54	27.0	5.0	4.0	4900/49	--
L	5.5	15.5	63	1.7	1.5	0.9	2/<2	--
SP	7.0	3.5	50	17.0	6.0	2.0	13,000/<2	--
L	4.0	13.0	87	2.8	2.4	2.5	490/130	--

TABLE 1. (CONTINUED)

Site	Flow rate (MGD)	Cl_2 residual (PPM)	Cl_2 contact time (min)	BOD (mg/1)	Turbidity (TU)	Suspended solids (mg/1)	Coliforms (total/fecal	Fecal strep
SP	6.0	4.0	54	17.0	5.0	2.0	790/13	--
L	5.0	17.0	73	3.2	1.8	2.4	2/2	--
SP	6.5	4.5	50	12.0	5.0	3.0	3300/79	--
L	ND	ND	ND	2.3	1.4	2.5	2/<2	--
SP	6.5	4.4	50	20.0	7.0	7.0	2300/230	--
L	6.0	14.0	58	2.6	1.6	1.9	23/2	--
SP	13.0	5.5	46	14.0	4.0	3.0	1100/22	--
L	4.5	16.0	80	3.4	2.6	3.0	9/<2	--
SP	8.5	5.6	39	13.0	5.0	4.0	170/<2	--
L	5.5	6.5	63	2.6	3.0	6.5	2/<2	--
SP	4.5	5.5	108	31.0	4.0	3.0	700/5	--
L	1.5	>20.0	ND	ND	ND	ND	2/<2	--
SP	6.25	6.5	94	36.0	7.0	8.0	7000/49	--
L	0.7	5.6	ND	ND	ND	ND	<2/<2	--
SP	8.0	5.5	74	32.0	7.0	6.0	1400/5	--
SP	7.0	5.5	84	19.0	5.0	4.0	330/2	--
L	3.0	9.0	ND	ND	ND	ND	<2/<2	--
SP	6.5	5.0	90	25.0	8.0	5.0	2200/11	--
SP	4.5	4.75	130	12.0	8.0	7.0	3300/17	2

TABLE 1. (CONTINUED)

Site	Flow rate (MGD)	Cl_2 residual (PPM)	Cl_2 contact time (min)	BOD (mg/1)	Turbidity (TU)	Suspended solids (mg/1)	Coliforms (total/fecal)	Fecal strep
L	ND	8.5	ND	16.2	2.2	4.0	<2/<2	<2
SP	8.0	4.25	74	ND	ND	ND	1300/230	13
L	2.4	8.0	ND	11.9	2.1	4.4	46/11	<2
SP	7.0	5.0	84	18.0	6.0	8.0	490/2	<2
L	6.2	14.0	56	29.4	ND	2.0	4/<2	<2
SP	4.75	5.0	126	10.0	10.0	1.0	1300/79	.8
L	5.5	9.0	63	32.4	2.1	3.6	<2/<2	<2
SP	7.0	4.5	84	14.0	7.0	9.0	790/11	<2
L	4.8	10.0	72	1.0	7.1	2.2	<2/<2	<2
SP	4.0	5.5	148	33.0	6.0	6.0	230/13	<2
L	3.8	6.0	98	ND	ND	7.0	<2/<2	<2
SP	8.5	4.75	70	27.0	9.0	12.0	1300/23	<2
L	3.5	9.0	100	3.5	ND	6.0	<2/<2	<2
SP	7.0	1.2	84	22.0	10.0	12.0	790/13	2
L	2.2	17.0	160	ND	1.1	1.0	<2/<2	<2
SP	7.0	5.1	86	17.0	8.0	8.0	1700/8	2
L	ND	12.0	ND	<1.8	2.1	0	<2/<2	<2
SP	7.0	5.0	86	29.0	20.0	30.0	7000/5	2
L	2.2	11.0	156	1.9	ND	0	<2/<2	<2

TABLE 1. (CONTINUED)

Site	Flow rate (MGD)	Cl_2 residual (PPM	Cl_2 contact time (min)	BOD (mg/l)	Turbid-ity (TU)	Suspended solids (mg/l)	Coliforms (total/fecal)	Fecal strep
SP	8.75	4.7	68	18.0	11.0	13.0	2300/4	23
L	1.9	10/0	166	7.0	3.8	<1.0	<2/<2	<2
SP	8.0	5.5	74	16.0	5.0	6.0	490/4	2
L	ND	2.5	ND	1.4	3.4	10.0	<2/<2	<2
SP	7.0	4.0	86	27.0	8.0	11.0	330/23	<2
L	ND	1.9	ND	3.0	11.0	1.0	<2/<2	<2

*ND = not determined

TABLE 2. ECHO, POLIO AND GROUP B COXSACKIEVIRUS ISOLATES FROM SAN PABLO AND LIVERMORE TREATMENT PLANTS RANKED ACCORDING TO FREQUENCY OF ISOLATION

Livermore		San Pablo	
Virus	No. Identified	Virus	No. Identified
Echo 7	177	Echo 7	310
Echo 11	76	Echo 8	108
Polio 3	67	CB 4	101
Echo 6	64	CB 2	94
Polio 2	57	Echo 1	84
CB 4	45	Echo 6	79
Echo 1	44	Echo 11	74
CB 2	42	Polio 3	58
Polio 1	35	Polio 1	50
CB 5	29	Polio 2	48
Echo 3	14	Echo 15	40
Echo 12, 13	11	CB 5	24
Echo 14, 24, 25	9	Echo 20	22
Echo 15, 20	8	Echo 17	17
Echo 19, CB 3	7	Echo 27	15
Echo 5	6	Echo 4, 5, 12	11
Echo 27	5	Echo 14, CB 1, 3	9
Echo 8, 17	3	Echo 13	8
Echo 4, 31, CB 1	2	Echo 19, 29, 33	6
Echo 18, 21, 22, 23, 29	1	Echo 3, 31	5
		Echo 22, 24	4
		Echo 25	3
		Echo 23	2
		Echo 9, 16, 21	1
Echo 2, 9, 16, 26, 30 32, 33, and CB 6 were not isolated.		Echo 2, 18, 26, 30, 32, and CB 6 were not isolated.	

Abbreviations: echo = echovirus, polio = poliovirus, CB = group B coxsackievirus

TABLE 3. ISOLATION OF GROUP A COXSACKIEVIRUS SEROTYPES IN FOUR TISSUE CULTURE CELL SYSTEMS

Coxsackievirus type	Cell system			
	RD	MFK	HFDK	BGMK
1	3	0	0	0
2	4	0	0	0
3	1	0	0	0
4	10	0	0	0
5	0	0	0	0
6	3	0	1	0
7	0	1	0	0
8	4	1	0	0
9	1	2	1	2
10	4	0	0	0
11	0	0	0	0
12	0	0	0	0
13	36	1	5	0
14	0	0	0	0
15	0	0	1	0
16	9	2	4	1
17	0	0	1	0
18	40	5	0	0
19	0	0	0	0
20	0	0	0	0
21	2	1	0	0
22	0	0	0	0
24	0	0	0	0
Total	117	13	13	3

TABLE 4. MICROBIAL QUALITY AND VIRUS FINDINGS IN WATERS ASSOCIATED WITH DISEASE OUTBREAKS

Outbreak Area	Water Source	Water Treatment	Sampling Period	Bacterial Indicators (Counts/100 ml)			Virus Isolation
				Total Coliforms	Fecal Coliforms	Fecal Strep	
Humboldt County*	Creek	Untreated	9/79	ND**	≥ 2400	ND	reovirus 2
					≥ 1100	ND	echo 11, 13 & 25 (19 liter sample)
Soda Springs*	Lake	Chlorination	1/80	21	5	ND	none
				<2	<2		(380 liter sample)
				<2	<2		
Soda Springs*	Lake	Prior to Chlorination	4/80	66	27	ND	reovirus 2 echovirus 14 (380 liter sample)
Grindstone* Indian Reservation	Wet Well	Untreated	6/80	64,000	30,000	2,300	Coxsackievirus B3 (380 liter sample)
Grindstone* Indian Reservation	Wet Well	Chlorination	7/80	13	<2	<2	none (380 liter sample)
Auburn	Private Well	Untreated	3/81	<2	<2	<2	none (380 liter sample)
Squaw Valley	Pond	Untreated	4/81	46	<2	12	none (570 liter sample)

*in the three outbreaks where viruses were isolated from subsequent water samples, Norwalk-like viruses were implicated as the etiological agent by RIA or IEM test of patient specimens.

**ND = not determined